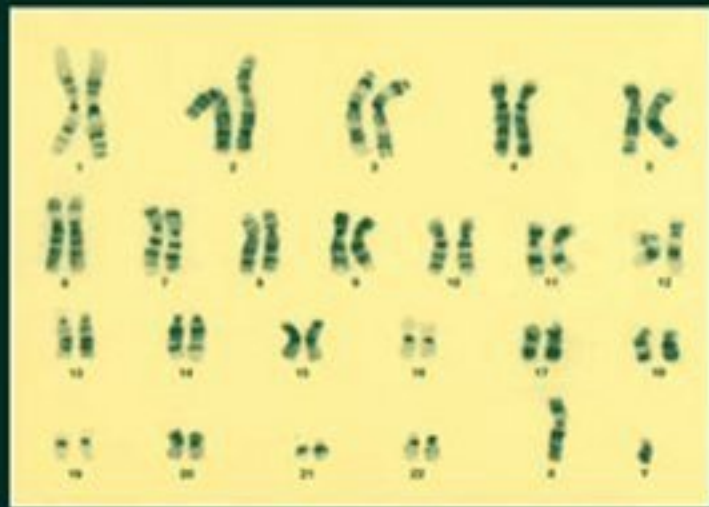


Bronya J.B. Keats
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Genetics and Auditory Disorders



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Genetics of Auditory Disorders

With 40 Illustrations



Springer

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Cover illustration: G-banded human male karyotype (46,XY), consisting of the normal complement of 46 chromosomes, including one X and one Y. This figure appears on p. 94 of the text.

Printed on acid-free paper.

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Production managed by Terry Kornak; manufacturing supervised by Erica Bresler.

Typeset by Best-set Typesetter Ltd., Hong Kong.

Printed and bound by Maple-Vail Book Manufacturing Group, York, PA.

Printed in the United States of America.

9 8 7 6 5 4 3

ISBN 0-387-98501-8

SPIN 10671976

Springer-Verlag New York Berlin Heidelberg

A member of BertelsmannSpringer Science+Business Media GmbH

Series Preface

The *Springer Handbook of Auditory Research* presents a series of comprehensive and synthetic reviews of the fundamental topics in modern auditory research. The volumes are aimed at all individuals with interests in hearing research including advanced graduate students, post-doctoral researchers, and clinical investigators. The volumes are intended to introduce new investigators to important aspects of hearing science and to help established investigators to better understand the fundamental theories and data in fields of hearing that they may not normally follow closely.

Each volume is intended to present a particular topic comprehensively, and each chapter will serve as a synthetic overview and guide to the literature. As such, the chapters present neither exhaustive data reviews nor original research that has not yet appeared in peer-reviewed journals. The volumes focus on topics that have developed a solid data and conceptual foundation rather than on those for which a literature is only beginning to develop. New research areas will be covered on a timely basis in the series as they begin to mature.

Each volume in the series consists of five to eight substantial chapters on a particular topic. In some cases, the topics will be ones of traditional interest for which there is a substantial body of data and theory, such as auditory neuroanatomy (Vol. 1) and neurophysiology (Vol. 2). Other volumes in the series will deal with topics that have begun to mature more recently, such as development, plasticity, and computational models of neural processing. In many cases, the series editors will be joined by a co-editor having special expertise in the topic of the volume.

Richard R. Fay, Chicago, IL
Arthur N. Popper, College Park, MD

Preface

The purpose of this volume in the *Springer Handbook of Auditory Research* series is to inform the reader about the many clinical forms of genetic hearing loss, the mutations that are responsible, and the functions of the proteins that are encoded by the mutant genes. This volume treats with equal importance the basic principles of genetics, the methods and techniques used in human molecular genetic studies, and the examples of how scientific results can be applied in patient care. An overview of the basic principles of genetics is provided in Chapter 1 (Keats and Berlin), together with citations to other chapters that expand on the topic. In addition, Chapter 1 ends with an extensive glossary of genetic terms with which some readers may be unfamiliar. Chapters 2 (Avraham and Hasson) and 5 (Giersch and Morton) describe the structure of genes and chromosomes, while Chapters 3 (Mueller, Van Camp, and Lench) and 5 explain the methodologies and the genomic tools used to find the chromosomal locations and identify disease genes. In Chapter 4, Nance and Pandya discuss the application of the laws of population genetics and models of genetic epidemiology to hearing loss, while Chapter 8 (Steel, Erven, and Kiernan) clarifies the relevance of mouse models to advancing understanding of hearing loss in humans. The clinical and genetic heterogeneity of genetic hearing loss are delineated in Chapters 6 (Griffith and Friedman) and 7 (Fischel-Ghodsian), with Chapter 6 providing an in-depth coverage of autosomal and X-linked forms of hearing loss and Chapter 7 concentrating on hearing loss due to mitochondrial DNA mutations. Finally, Chapter 9 (Arnos and Oerlich) illustrates the role of the genetic counselor in communicating knowledge about hearing loss to the patient, thus translating the findings of medical science into clinical care.

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April, 2001

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1

Introduction and Overview: Genetics in Auditory Science and Clinical Audiology

BRONYA J.B. KEATS and CHARLES I. BERLIN

1. Introduction

The prevalence of genetic hearing loss varies among studies, but overall at least 50% of hearing loss is genetic (Morton 1991; Marazita et al. 1993; Nance and Pandya, Chapter 5). Furthermore, hearing loss that follows an environmental insult (such as infection, acoustic trauma, exposure to ototoxic drugs) is likely to be influenced by genetic factors. Hearing loss is, in fact, one of the most common genetic disorders, and a genetic etiology must be considered for all patients with hearing loss. Identifying the underlying causes of genetic hearing loss provides the clinical audiologist with useful tools for diagnosis, prognosis, and, potentially, treatment for hearing impaired patients. It also demonstrates that these genes, and the proteins they encode, are critical for normal development of the auditory system. Thus, all investigators interested in the auditory sciences, as well as patients in audiological clinics, benefit from genetic studies of hearing loss.

Approximately one child in a thousand is born with profound hearing impairment, and at least 70% have no associated anomalies (Gorlin et al. 1995). Analysis of data collected by Gallaudet University in the 1969–1970 Annual Survey of Hearing Impaired Children and Youth estimated that genetic causes explained the hearing impairment in 50.7% of the children (Nance et al. 1977), while analysis of the survey data collected two decades later (1988–1989) estimated a genetic etiology in 62.8% of cases (Marazita et al. 1993). A major reason for the increase in the proportion of profound hearing impairment due to genetic factors was the reduction in cases due to environmental causes (in particular, rubella).

Adult-onset hearing loss is also a significant health problem, with 14% of individuals between the ages of 45 and 64, and 30% of those older than 65 years, having hearing problems (Hotchkiss 1989). Sill et al. (1994) collected extensive population data in an attempt to delineate the causes of later-onset hearing loss. The data strongly suggested a genetic etiology in a large percentage of the participants, but a precise estimate could not be determined.

Awareness that inheritance is important in hearing impairment can be traced to the sixteenth century (see Nance and Pandya, Chapter 5, for a summary of these early studies), and more recently, Konigsmark (1969), Konigsmark and Gorlin (1976), Fraser (1976), and Gorlin et al. (1995) provided comprehensive reviews of hereditary hearing impairment and associated abnormalities emphasizing the extensive clinical and genetic variability. Estimates of the number of genes causing inherited hearing impairment (Stevenson and Cheeseman 1956; Chung and Brown 1970) varied depending on the size of the survey and the genetic heterogeneity of the particular population studied (Morton 1991).

One purpose of this volume in the Springer Handbook of Auditory Research series is to inform the reader about the many clinical forms of genetic hearing loss, the mutations that are responsible, and the functions of the proteins that are encoded by the mutant genes. However, the editors and authors view with equal importance the basic principles of genetics, the methods and techniques used in human molecular genetic studies, and the examples of how scientific results can be applied in patient care. Chapters 2 (Avraham and Hasson) and 3 (Giersch and Morton) describe the structure of genes and chromosomes, while Chapters 3 and 4 (Mueller, Van Camp, and Lench) explain the methodologies and the genomic tools used to find the chromosomal locations and identify disease genes. In Chapter 5, Nance and Pandya discuss the application of the laws of population genetics and models of genetic epidemiology to hearing loss, while Chapter 8 (Steel, Erven, and Kiernan) clarifies the relevance of mouse models to advancing understanding of hearing loss in humans. The clinical and genetic heterogeneity of genetic hearing loss are delineated in Chapters 6 (Griffith and Friedman) and 7 (Fischel-Ghodsian), with Chapter 6 providing an in-depth coverage of autosomal and X-linked forms of hearing loss and Chapter 7 concentrating on hearing loss due to mitochondrial DNA mutations. Finally, Chapter 9 (Arnos and Oerlich) illustrates the role of the genetic counselor in communicating knowledge about hearing loss to the patient, thus translating the findings of medical science into clinical care.

An overview of the basic principles of genetics is provided in this chapter, together with citations to other chapters that expand on the topic. In addition, this chapter ends with an extensive glossary of genetic terms with which some readers may be unfamiliar. More comprehensive coverage of human genetics may be found in textbooks such as Nussbaum et al. (2001) and Gelehrter et al. (1998). But before getting to the genetics of hearing loss, the phenotype requires both a definition and a discussion.

1.1 Defining and Quantifying the Hearing Loss Phenotype

The findings that result from a clinical examination are an individual's phenotype. They may include biochemical, physiological, and morphological characteristics. This phenotype can result from genetic factors, environmental factors, or a combination of both. The genetic factors are the individual's genotype. Hearing loss may be just one of the features of the phenotype, in which case the disorder is known as a syndrome; if no other clinical anomalies are apparent, the hearing impairment is called nonsyndromic. Gorlin et al. (1995) describe 30 syndromes in which hearing loss is associated with abnormalities of the external ear, 40 with the eye, 87 with the musculoskeletal system, 23 with the kidney, 56 with the skin, 63 with the central nervous system, 51 with endocrine and metabolic conditions, 12 with chromosomal anomalies, 8 with oral and dental problems, and 35 with miscellaneous disorders, as well as 22 forms of nonsyndromic hearing impairment. The phenotypes and genotypes of many of these syndromes, as well as those of nonsyndromic hearing loss, are described in Chapters 6 and 7. The reader is able to keep updated on new hearing loss genes by accessing the Hereditary Hearing Loss Homepage (URL: <http://dnalab-www.uia.ac.be/dnalab/hhh/>).

The hearing loss phenotype has been most commonly quantified by obtaining a pure tone air conduction audiogram. The work that led to the development of the equipment necessary to make these measurements was done over 70 years ago. Georg von Békésy had just reported his theory on the traveling wave in the cochlea and its role in frequency analysis in 1929 (see Békésy 1960), when Crowe et al. (1934) published their paper showing that high-frequency hearing loss was associated with loss of hair cells at the base of the cochlea. The convergence of these independent lines of investigation led to considerable excitement in the field. Up to that time, hearing loss had been estimated with mechanical devices such as a monochord (a bow-activated, carefully calibrated string), whistle (Bunch 1943), and, of course, a tuning fork. What was unmistakable, however, was that Crowe et al. (1934) had used what was then a revolutionary instrument to quantify hearing loss. Their tool was the Western Electric 1-A audiometer, a battery driven device with an oscillator that produced pure tones up to 16,384 Hertz (Hz), and an interrupter, attenuator, and black bakelite earphones, whose output verification was difficult to quantify at the time. However, the voltage to the terminals of the phones was quite constant, and hearing losses were expressed both in decibels (dB) of attenuation and dB relative to what was thought to be normal sensitivity at octave intervals from 64 to 16,384 Hz. Thus, Crowe et al. (1934) were able to quantify hearing loss in dB, although at the time they were less interested in clinical issues than in harvesting bones with quantifiable hearing loss. It was some time before norms were developed, but this early 1930s electronic audiome-

ter predated audiology as it is practiced today and was the cutting edge of auditory assessment in humans.

The pure tone air conduction audiogram is at best only a rudimentary tool for measuring hearing loss. Today's physiologically based audiological battery should include tympanometry, middle ear muscle reflexes, otoacoustic emissions, speech audiometry, and Auditory Brainstem Response/Electrocochleography (ABR/EcochG). Auditory Brainstem Response and Electrocochleography are computer-controlled averaged evoked responses that delineate synchronous neural discharges, usually following a brief auditory pulse. They correlate extremely well with hearing, although they are not themselves hearing tests (Hood and Berlin 1986). Understanding why these additional tests are essential for fully accurate phenotyping requires review of the following basic principles of auditory physiology.

The five major electroacoustic events in the cochlea are:

1. The endocochlear potential
2. The cochlear microphonic
3. The compound action potential
4. The summing potential
5. Otoacoustic emissions

Selective abnormalities in one or a number of these elements can lead to 120 different permutations of these five events, although current level of knowledge can rule out a number of impossible sequences. For example, absence of endocochlear potential leads to an absence of all the other electrical events, including the otoacoustic emissions. It is therefore impossible, as far as we can tell today, to have an absent endocochlear potential and recordable otoacoustic emissions, or any of the above events. Removing such impossible sequences reduces the number of possible permutations to about 70.

Nowadays, parts of the organ of Corti can be assessed separately. For example, normal otoacoustic emissions indicate that the endocochlear potential, the middle ear, and the (primarily) outer hair cells are working well. ABR and EcochG provide information about the synchrony and integrity of the inner hair cells and eighth nerve, as well as confirming neural synchrony after the discharge of the primary neurons. Thus, these tests and their successors should be used not just in identifying the presence or absence of tumors, but also in assessing the integrity of the auditory nervous system and helping develop methods to categorize various genetic hearing losses audiotically. The same pure-tone audiogram might be observed in one patient with a genetically based loss of outer hair cells, and another with normal outer hair cells, but disturbed neural synchrony (Berlin et al. 1993; Starr et al. 1996). However, etiology and genotypes are quite different, and classifying both patients according to their pure-tone audiograms alone would not be adequate for genetic analysis. A more precise categorization of the hearing loss can be made by testing otoacoustic emissions,

which allow outer hair cell cochlear function to be assessed somewhat independently of inner hair cell and neural function. Recent studies suggest that emissions may be helpful in discriminating between those who carry certain mutations that cause recessive nonsyndromic sensorineural hearing impairment and those who do not (Morell et al. 1998). Additionally, by performing tests for middle ear muscle reflexes, ABR, and otoacoustic emissions, a patient with hearing loss caused by abnormal hair cells can be distinguished from one with normal hair cells (Berlin et al. 1996). Patients with normal outer hair cells do not usually benefit from hearing aids in the frequency ranges where their hair cells, and presumably their emissions, are normal.

It is important to remember that the etiology of hearing loss may not be the same, even for members of the same family. One or more members of a family may have an acquired hearing loss that mimics that of members with genetic hearing loss. Such phenocopies in a family are common if the only test that is done for phenotyping is a pure tone audiogram. This practice should be discouraged, and all family members should have at least tympanometry, middle ear muscle reflexes, otoacoustic emissions, speech audiometry, pure tone audiometry by air and bone, and speech discrimination testing, if at all possible.

A poignant example (uncovered as part of our genetic studies) is a family in which several members were reported to show marked hearing losses based on pure-tone audiometry. However, by adding tympanometry, reflexes, emissions and speech audiometry to the phenotyping battery, one of the patients, a 36-year-old female who had been wearing hearing aids since she was 7 years old, volunteered a pure-tone average hearing loss of 65 dB, but had normal otoacoustic emissions, normal reflexes, a Speech Reception Threshold of 20 dB, and 92% discrimination at only 40 dB HL. Experienced audiologists recognize the anomaly of a 65 dB hearing threshold with a 20 dB Speech Reception Threshold. The patient's hearing aids were in her ears, but were never turned on. Further inquiry revealed that she had been hospitalized for conversion hysteria, and she admitted to adopting the hearing loss so that she "would belong." As discussed later in this chapter, incorporating this individual into a linkage study based on her pure tone audiogram would have resulted in inaccurate conclusions.

The audiologist needs to be sensitive to the importance of accurate and comprehensive phenotyping and is urged to obtain more than simple pure-tone audiometry to assist the geneticist in both research studies and clinical service.

1.2 Inheritance Patterns of the Phenotype

The human genome consists of 24 different types of chromosomes in the nucleus of a cell. They are designated 1–22 (autosomes), X and Y (sex chromosomes). An offspring inherits one set of chromosomes from each parent,

providing a total of 46 chromosomes (22 autosomal pairs plus XX or XY) in the nucleus of a cell. If both parents transmit an X chromosome, the offspring is female (XX); if the father transmits a Y chromosome, then the offspring is male (XY). The distribution of family members with hearing loss may be consistent with autosomal or X-linked inheritance, which may be further categorized as dominant or recessive. The symbol assigned to a non-syndromic form of hearing loss reflects the mode of inheritance. DFNA* and DFNB* (*the asterisk represents a number that is assigned when the gene for the hearing loss is localized to a chromosome) indicate autosomal dominant and autosomal recessive, respectively, while DFN* means X-linked. The number does not reflect the chromosomal location; *DFNB7*, for example, simply means that the gene for this form of autosomal recessive hearing loss was the seventh to be localized (the *DFNB7* gene is on chromosome 9). If the pattern of inheritance is consistent with maternal transmission, the location of the gene may be the mitochondrial genome (Fischel-Ghodsian, Chapter 7). For hearing loss, there is general agreement that the pattern of inheritance is autosomal recessive in about 77% of cases, autosomal dominant in about 22%, and X-linked or mitochondrial in the remainder (Gorlin et al. 1995).

An individual has pairs of chromosomes and, therefore, pairs of genes and pairs of genetic markers (fragments of DNA that have unique chromosomal locations). The chromosomal location of a gene or genetic marker is its locus. The different forms of genes and genetic markers are called alleles; thus, at each locus an individual has two alleles, and this pair of alleles is the genotype. The genotype of the individual is homozygous if the two alleles are the same, and heterozygous if they are different.

1.2.1 Autosomal Dominant Inheritance

The pattern of inheritance is probably autosomal dominant if individuals in each generation are affected; both males and females are equally likely to be affected. Affected individuals are usually heterozygotes with one normal and one deleterious copy of the gene for the disorder, and each offspring of an affected individual has a 50% chance of inheriting the deleterious allele. However, knowing the genotype may not be adequate in predicting the phenotype. There may be variable expression among affected individuals, and some who must have the deleterious allele (because, for example, they have an affected parent and an affected child) may show no phenotypic signs.

Penetrance is defined as the probability of expressing features of the phenotype given the presence of a particular genotype. For a dominant disorder, if 70% of individuals who have the deleterious allele show some phenotypic expression of the disorder, then the penetrance value is 0.7. Penetrance may also be age dependent, such as with adult-onset hearing loss. In this case, the probability that a person who has the deleterious allele will

have hearing loss increases with age. Thus, penetrance may be close to zero at 10 years of age, 0.3 at 20 years of age, 0.7 at 30 years of age, and 1.0 at 50 years of age. The degree of penetrance needs to be taken into account when calculating recurrence risks for individuals who have relatives with an autosomal dominant disease. Incomplete penetrance and/or variable expression among family members suggests that other genes and environmental factors are contributing to the phenotype.

An offspring with an autosomal dominant disorder may sometimes have two unaffected parents. As discussed above, this situation is observed with incomplete penetrance (a value less than 1.0). If, in addition, the affected offspring has no affected relatives, then the possibility of a new mutation must be considered. For example, more than 80% of cases of achondroplasia (short-limbed dwarfism) are new mutations in germ cells (sperm or ova). Occasionally, unaffected parents have more than one offspring with a dominant disorder. A likely explanation in this situation is germline mosaicism, which occurs if the mutation arose during the embryonic life of one of the parents in a cell destined to be a germ cell. As a result, some (or possibly all) of the germ cells carry the mutation.

1.2.2 Autosomal Recessive Inheritance

The mode of inheritance of a disorder is autosomal recessive if the abnormal phenotype is expressed only in individuals who have two copies of the deleterious allele. Heterozygotes are not affected and are often called carriers. Assuming complete penetrance, the probability that a child of carrier parents will be affected is one quarter; the probability that an unaffected sibling is a carrier is two thirds (Fig. 1.1). If a hearing impaired child of normal hearing parents has an affected sibling or the parents are related, then the etiology is most likely to be a deleterious gene with the mode of inheritance being autosomal recessive. Alternatively, if a known environmental insult such as an infectious agent or a drug is documented, then the presence of a deleterious gene is less likely. However, the possibility of recessive inheritance must always be considered. Note that family history is quite likely to be negative with a recessive disorder because the abnormal allele may be passed from one (unaffected) carrier to the next for many generations before a couple, who by chance both carry the same deleterious allele, has an affected child. Recessive inheritance probably explains most cases of profound sensorineural hearing loss when family history is negative, and there is no known environmental factor that could be responsible.

It is not uncommon for deaf individuals to marry one another. However, the underlying causes of their deafness may be different, even though both may have recessive deafness. If both are homozygous for the same deleterious allele, then all their children will also be homozygous for this allele. However, if deleterious alleles for different genes are responsible for the

Alleles: H, D
Genotypes: HH, HD (or DH) - Hearing
DD - Deaf

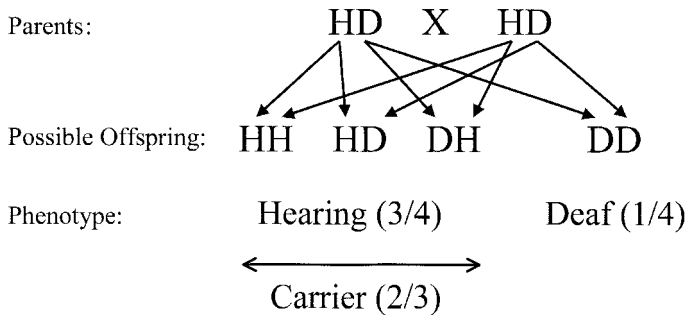


FIGURE 1.1. Recessive deafness. The probabilities (in parentheses) of each of the possible offspring phenotypes and genotypes are shown for parents who are both carriers.

deafness in the spouses, then all their offspring will probably be heterozygous for two deafness genes, and not homozygous for either of them. Thus, deaf spouses, who may each have many deaf relatives, can have offspring with normal hearing.

1.2.3 X-linked Inheritance

Males have both an X and Y chromosome; females have two X chromosomes. Thus, a necessary condition for X-linked inheritance is no father-to-son transmission because a son receives his X chromosome from his mother. If a disorder has an X-linked dominant pattern of inheritance with complete penetrance, then all daughters of affected fathers are affected, and each child of an affected mother has a 50% chance of being affected. In the case of an X-linked recessive trait, the majority of affected individuals are male because they have only one X chromosome. Females have two X chromosomes and may be carriers, but are unlikely to be affected. Sons of a carrier mother each have a 50% chance of inheriting the abnormal allele, while daughters have a 50% chance of being carriers. Note that, although females have two X chromosomes in a cell, in general one of the two is randomly inactivated early in embryonic development. Thus, females are mosaic, with each cell having one or the other X chromosome active.

1.2.4 Mitochondrial Inheritance

Mitochondria are small organelles that are located within the cytoplasm of a cell; they are independent of the nucleus and have their own DNA

(mtDNA). A process called oxidative phosphorylation (OXPHOS) takes place in the mitochondria and is responsible for energy (ATP) production in the cell. Each mitochondrion has multiple copies of mtDNA and each cell contains several hundred mitochondria, and thus many copies of mtDNA. The mutation rate is ten times higher in mtDNA than in nuclear DNA, and cells may contain both mutant copies and normal copies, a condition known as heteroplasmy. If the normal copies can successfully provide the energy requirements of the cell, then normal function will be retained. However, different cell types have differing energy requirements. The organs with the most demand for energy are the skeletal muscle, heart, eye, ear, and brain. Thus, the typical symptoms found in mitochondrial disorders are muscle weakness, nervous system disorders, visual problems, hearing loss, and dementia.

The mtDNA molecule is circular and consists of about 16,000 base pairs. Mitochondria are maternally inherited, so the expected family history for a mitochondrial disorder is that all children of an affected mother are affected, and children of an affected father are never affected. However, because of heteroplasmy, the mother and siblings of an affected individual may show only minor symptoms, or none at all. Mitochondrial disorders that involve hearing loss are discussed by Fischel-Ghodsian (Chapter 7).

2. The Genetic Material

Each chromosome consists of a single molecule of deoxyribonucleic acid (DNA), which has a double-helical structure. DNA is composed of a sugar-phosphate backbone and four bases: adenine (A) and guanine (G) are purines, and thymine (T) and cytosine (C) are pyrimidines. The double helix is formed through the pairing of A with T, and C with G, and the bases are held together by hydrogen bonds. Thus, knowing the sequence of bases on one DNA strand automatically gives the sequence on the other strand. This precise pairing means that DNA can replicate by separation of the two strands, followed by each strand serving as a template for a new complementary strand.

The set of 24 chromosomes has three billion base pairs, of which 1% to 2% make up the estimated 35,000 human genes. Genes consist of both coding sequences (exons) and intervening sequences (introns), and it is the exons that encode the amino acid sequence of the protein product. The connecting link between the gene (DNA) and the protein is messenger ribonucleic acid (mRNA). The sequence of bases in the exons is called the complementary DNA (cDNA) sequence because it can be synthesized using the mRNA as a template. The three major differences between DNA and RNA are: (1) DNA is double-stranded, RNA is single-stranded; (2) the

sugar in DNA is deoxyribose, the sugar in RNA is ribose; (3) thymine in DNA is replaced by uracil (U) in RNA.

There are 20 different amino acids, and each set of three bases in the mRNA constitutes a codon, which specifies either a particular amino acid, or a stop signal. This correspondence is called the genetic code. Thus, the amino acid sequence of a protein can be deduced from the cDNA (or mRNA) sequence. In Chapter 2, Avraham and Hasson provide a succinct discussion of the organization of chromosomes and genes and the flow of information from DNA to RNA to protein. Avraham and Hasson also delineate how different types of mutations result in abnormal gene expression or gene products.

Less than 2% of DNA codes for proteins. Some of the noncoding sequence seems to be important for control of gene expression, but a large amount has no known function. Repetitive sequences are found dispersed throughout the genome, with the most common being the *Alu* sequence, which is about 300bp in length and occurs about 500,000 times in the human genome. Any DNA segment of 30,000bp is likely to contain an *Alu* sequence, but whether these sequences serve any function is not known.

Chromosome morphology is generally defined by the position of a constricted region called the centromere, which separates the p (or short) arm from the q (or long) arm. If the centromere is near the middle, the chromosome is metacentric, and if it lies near the end (telomere), the chromosome is acrocentric. The telomeres are specialized structures that are thought to protect chromosome ends from degradation. Chromosomes can generally be distinguished from one another based on size and position of the centromere, but they can also be differentiated in terms of banding patterns that are obtained when the chromosomes are stained with certain compounds. The resulting set of dark and light bands is numbered so as to provide coordinates for specific regions of the chromosome.

Abnormal chromosomes are probably responsible for more than half the spontaneous abortions, and they are also often found in children with congenital malformations. Giersch and Morton (Chapter 3) provide a comprehensive review of the many types of chromosomal abnormalities that occur, and particularly emphasize those in which the phenotype includes hearing loss.

3. Genes in Populations

Allele and genotype frequencies in a population depend on factors such as mating patterns, population size, mutation, migration, and selection.

By making specific assumptions about these factors, Hardy (1908) and Weinberg (1908) independently formulated what is now known as the Hardy–Weinberg law. This fundamental principle of population genetics states that, if there are two alleles at a single autosomal locus with population frequencies p and q , then, for a random mating population in equilibrium, the frequencies are p^2 and q^2 for the two homozygous genotypes and $2pq$ for the heterozygous genotype. Moreover, these frequencies remain the same from one generation to the next. Thus, if the frequency of a recessive disorder in a population is 1 in 10,000 (that is, $q^2 = 0.0001$), then $q = 0.01$ (and $p = 0.99$ because $p + q = 1$), and the frequency of carriers of the deleterious gene can be calculated as $2pq$, which is 1 in 50.

The Hardy–Weinberg law does not hold if mating is not random in the population. For example, if mating is consanguineous (between relatives) or assortative (between individuals with the same phenotype, such as deafness, as discussed by Nance and Pandya in Chapter 5), then the frequency of heterozygotes is less than $2pq$, and the frequencies of homozygotes are increased. Also, evolutionary forces such as mutation, migration, and selection change allele frequencies. However, Hardy–Weinberg equilibrium at an autosomal locus will be restored in one generation after the force is no longer disturbing the allele frequencies.

Now consider two autosomal loci, both with two alleles (A_1, A_2 and B_1, B_2). During meiosis (cell division that results in the haploid germ cells), crossing-over may take place between a pair of chromosomes. Suppose that one chromosome has the A_1 allele at the first locus and the B_1 allele at the second locus, while the other chromosome has A_2 and B_2 , respectively. If crossing-over occurs, then the haplotype (the set of alleles on the same chromosome) in a germ cell may be A_1B_2 , or A_2B_1 . If crossing-over does not occur between these two loci, then all the germ cells will have the haplotypes, A_1B_1 , or A_2B_2 . A recombination event is a result of crossing-over that can be observed in the offspring and is explained in detail by Mueller, Van Camp, and Lench in Chapter 4.

Linkage disequilibrium is present if the product of the population frequencies of the A_1B_1 and A_2B_2 haplotypes is not equal to the product of population frequencies of the A_1B_2 and A_2B_1 haplotypes. In general, this means that not enough recombination events between the two loci have yet taken place in the population to equalize these products. For two loci that are close together, many thousands of generations may be required to reach equilibrium at the two loci considered jointly.

The estimation of the frequency of recombination events is the basis of linkage analysis, which is a method for mapping genes to chromosomes. To detect linkage, family data (not population data) must be analyzed. Two loci are said to be linked if the frequency of recombination events among offspring is less than 50%. If recombination events are very rare

between a disease gene and a genetic marker, then it is likely that the chromosomal location of the disease gene is very close to that of the genetic marker.

3.1 *Localizing and Identifying Genes*

One goal of genetic research is to localize, identify, and establish the base sequence of all genes. Genetic linkage analyses of families in which some members have an inherited disorder provide the chromosomal location of the deleterious gene. A linkage study may require typing hundreds of genetic markers before the disease gene is localized, but once this step is accomplished, research to identify candidate genes in the region begins. For recessive hearing impairment, affected individuals whose parents are related are likely to have the same homozygous genotype. Thus, the search for the location of the gene may be accelerated by screening pooled DNA samples from these individuals and selecting markers for which they are all homozygous for the same allele (Sheffield et al. 1994). If pooling is feasible, the number of samples that need to be typed can be reduced dramatically. For example, Scott et al. (1995) used this approach in their study of *DFNB1* Bedouin families (Mueller, Van Camp, and Lench, Chapter 4; Griffith and Friedman, Chapter 6). When the gene is identified and mutations are detected, diagnostic tests that examine the DNA within the gene itself are applicable to all individuals. Mueller, Van Camp, and Lench describe the methodology used to identify genes and detect mutations in Chapter 4.

As well as providing the approximate location of a disease gene, linkage analysis permits more precise genetic counseling (Arnos and Oerlich, Chapter 9). For example, by examining the transmission in a family of a closely linked genetic marker, relatives of individuals affected with a recessive disorder can be informed as to whether they are likely to be carriers.

The development of high-resolution genetic and physical maps, together with the construction of genomic and cDNA libraries, and the availability of sequence databases for many species, provide the tools for finding genes once they have been localized by linkage analysis. Giersch and Morton (Chapter 3) describe some of these tools. Detecting a mutation in a candidate gene in affected individuals may mean that the search for the gene responsible for the hearing impairment is over, particularly if that mutation is not found in a sample of unaffected individuals. However, DNA sequence varies from one individual to another, so a sequence difference may not necessarily make the gene deleterious; it may simply reflect normal variation. Some mutations, though, such as a deletion or insertion of several bases, or a point mutation (single base change)

that gives rise to a codon that does not correspond to an amino acid (e.g., a stop codon), are very likely to be causal (Avraham and Hasson, Chapter 2).

3.2 *Heterogeneity*

The underlying cause of most forms of hereditary hearing loss is a mutation in a single gene. Results from linkage studies of several families are often pooled to increase the probability that the correct location has been found for the gene. However, the gene in one family may be different from that in another (e.g., at least nine different genes cause Usher syndrome). This is known as locus heterogeneity. In this situation, pooling families will hinder rather than help the analysis. Studying endogamous populations or large pedigrees minimizes the chance of locus heterogeneity, but does not necessarily eliminate it.

A phenotype such as nonsyndromic hearing impairment is caused by many different genes, and the etiology may not be the same even for affected members of the same family. In some cases, auditory testing (particularly if it covers more than pure tone air conduction audiometry) may detect phenotype differences among members of the same kindred. Such findings may be critical for linkage studies. For example, in the family studied by Vahava et al. (1998) auditory testing showed that the hearing impairment in one member was different from that in the other members. Knowing that this individual was a phenocopy (same clinical phenotype, but different etiology) facilitated the linkage analysis.

Unlike locus heterogeneity, allelic heterogeneity does not confound linkage studies. In this case, different alleles (that is, different mutations in the same gene) are responsible for the phenotypes, which are generally similar. However, different alleles do not necessarily result in the same phenotype, and the mode of inheritance may even be different. For example, most mutations in the human myosin VIIa gene (*MYO7A*) cause Usher syndrome type IB, but a few result in recessive nonsyndromic hearing loss, and others in dominant nonsyndromic hearing loss.

4. Mouse Models

Finding a mutation that probably makes a gene deleterious is a critical step, but it does not prove that the defect actually causes the hearing impairment. However, if a transgenic or knockout mouse is hearing impaired, then the argument that the defect causes the abnormal phenotype is convincing. To obtain a transgenic mouse, copies of the gene are injected into mouse

oocytes just after fertilization. The oocytes are then implanted into a foster mother whose uterus has been prepared for pregnancy by treatment with hormones. The transgene may incorporate anywhere in the genome, and several copies (sometimes as many as 200 copies) are usually found at a single location. In general, between 10% and 30% of the progeny are found to have the injected gene in their germline DNA, and can therefore pass it on to their offspring, thus allowing the development of a colony of transgenic mice.

The construction of a knockout mouse is a much more controlled and precise experiment than generating a transgenic mouse. The first step is to replace the normal copy of the gene with a copy containing the mutation of interest. This procedure is carried out in embryonic stem (ES) cells derived from mouse blastocysts (an early stage of embryonic development) and grown in tissue culture. The mutant gene may insert anywhere in the genome, but only the cells in which the normal copy is replaced by the mutant copy are selected for the next step. The selected ES cells are then injected into a recipient blastocyst, which is implanted into a foster mother. The resulting offspring will be chimeric, meaning that some of their cells are derived from the ES cell line, and some are from the recipient blastocyst. Mating experiments may then be set up in order to determine if the ES cells have contributed to the germline and to generate a colony of knockout mice. Knockout mice provide excellent animal models for studying the effects of gene mutations associated with human disorders.

Because of the high degree of similarity (orthology) of genes in humans and mice, studies of mouse mutants have made many valuable contributions to human disease gene identification (Meisler 1996), and hearing impairment is no exception (Brown and Steel 1994). Major advantages of using the mouse for finding disease genes are the ability to set up specific matings, and the relative ease of obtaining large numbers of informative progeny to localize the genes by linkage analysis. A relevant example is human *USH1B* and the mouse deafness mutant *shaker-1* (*sh1*), which were hypothesized to be caused by mutations in orthologous genes because they had been mapped by linkage to a conserved region on human chromosome 11q13 and mouse chromosome 7. This hypothesis was proven to be correct when Gibson et al. (1995) showed that the *sh1* gene encoded myosin VIIa, and Weil et al. (1995) found mutations in the human *MYO7A* gene in Usher type IB patients soon thereafter. Note that symbols for human genes are uppercase, while those for mouse genes are lowercase. Thus, the *USH1B* gene is *MYO7A*, and the *sh1* gene is *myo7a*. Steel, Erven, and Kiernan (Chapter 8) cover the application of mouse models to studies of human hearing impairment.

Mapping and sequencing of genes in non-mammalian species is also proving to be useful for studies of human diseases. In particular, the

zebrafish is a remarkably useful model for studying development and genetics (Driever and Fishman 1996). A major advantage of the zebrafish is that the embryo is transparent, which means that subtle developmental phenotypes can be studied *in vivo*. Additionally, comparative mapping demonstrates that the human and zebrafish genomes have regions in common (Woods et al. 2000), and function of orthologous genes can often be analyzed more effectively in zebrafish than in other species.

5. Genetic Counseling

The importance of imparting state-of-the-art scientific knowledge concerning diseases to patients cannot be overemphasized. At the same time, this information must be provided accurately and in a way that does not leave the patient with unrealistic or false hopes for diagnosis or therapy. A genetic counselor is trained to provide this information. Arnos and Oerlich (Chapter 9) provide a clear and thoughtful discussion of the critical role of the genetic counselor. Genetic counseling attempts to answer questions concerning etiology (e.g., Why is our child hearing impaired?) and risk (e.g., What is the chance that we will have a hearing impaired child?). These questions may come from the parents of a child with hearing impairment, from a hearing impaired individual who wants to have a child, or from a person who has hearing-impaired relatives. As well as answering these questions, the genetic counselor can provide information concerning the availability of genetic diagnostic testing. A critical part of genetic counseling is making sure that the patient understands the information being conveyed. It is essential that there be no communication barriers, and genetic counseling of hearing impaired individuals must be done in an appropriate manner using visual material and/or a sign language interpreter.

The possibility that the hearing loss may be part of a syndrome must be considered, and the genetic counselor again has the knowledge to inform the patient or parents. For example, a child may have Jervell and Lange-Nielsen syndrome, or Usher syndrome (Griffith and Friedman, Chapter 6), and testing for these syndromes may be appropriate. In particular, biotinidase deficiency can cause a form of genetic deafness that is preventable if the deficiency is detected and treated. Thus, testing hearing impaired infants for biotinidase deficiency in states where newborn screening for biotinidase deficiency is not mandatory should be recommended.

6. Gene Therapy

Gene therapy is the term used to describe a method of treatment of a human disorder in which a gene is transferred into the cells of the particular organ that is affected. A normal copy of the gene that is deleterious in

the patient is packaged into a vector and introduced to the cells. The gene is not incorporated into the germline cells. The purpose of gene therapy is to treat the patient, not to change the genetic material that is passed to the next generation. Many research studies are in progress to determine effective vectors and suitable approaches for delivering genetic material to specific tissues. Certain classes of viruses may be used as vectors, and ongoing investigations are exploring their potential in gene therapy. Nonviral strategies such as coating the gene in a lipid layer are also being developed. Although still in its infancy, gene therapy holds great promise for future treatment of genetic disorders, including hearing impairment. Cook et al. (2000) provide a summary of ongoing gene therapy research that targets the inner ear.

7. Summary

Genetic studies are resulting in the identification of genes for many disorders. In theory, once a disease gene is identified, diagnostic tests can be developed to detect mutations in individuals who may have the disease. Offering such diagnostic tests can be beneficial if one or two mutations are responsible for most cases. For many genes, however, unrelated affected individuals are likely to have different mutations, making detection a time-consuming and expensive task, especially if the gene is large. Moreover, finding a sequence difference does not necessarily mean that the responsible mutation has been found; sequence variation among individuals is common and very few of the variants are associated with a disorder. Thus, setting up diagnostic tests that are commercially available may not be economically feasible. On the other hand, once a mutation is identified in one family member, related individuals can be tested for this mutation. This can be beneficial for: (1) presymptomatic diagnosis in later age at onset disorders; (2) detecting the presence of the deleterious gene when penetrance is not complete, meaning that unaffected individuals may have the gene; and (3) identifying carriers of the gene when the inheritance pattern is recessive.

Many ethical issues must also be considered. Should molecular diagnostic testing be offered routinely if the result will not provide an alternative approach to treatment and management of the disorder? What are the psychological effects of presymptomatic genetic testing? Should parents be able to have their children tested for the presence of a mutation for which the age at onset of symptoms is after they reach adulthood? What is the obligation to other family members if a deleterious gene is detected? Remember, genes are transmitted from one generation to the next, and the results for one individual are pertinent to many relatives.

7.1 *A Look to the Future*

Routine molecular diagnostic testing for many of the genes causing hearing impairment is likely to be available within the next few years, and genetic testing may become the method of choice for newborn hearing screening. Although the development of effective therapies based on gene identification is still in its infancy, promising new advances made possible by research are arising at a rapid rate, and the potential for developing molecular intervention strategies as a treatment, and perhaps cure, is encouraging. A prerequisite, though, is determining the normal function of proteins encoded by hearing impairment genes.

Knowing the gene mutation that is responsible for hearing impairment in an individual provides information that is relevant to all family members. Based on this information, they may choose to be tested in order to help in making reproductive decisions. In addition to genetic tests, a desirable goal is to be able to predict the phenotype based on the genotype, and vice versa. However, defining the precise phenotype and providing an accurate prognosis based on the mutation is not yet straightforward. Physiologic indices that correlate with the presence of a mutation are valuable phenotypic measures that will enhance our understanding of the function of the normal gene product (Hood 1998; Huang et al. 1996, 1998; Liu and Newton 1997; Morell et al. 1998). The development of such measures that accurately define the phenotype, together with the application of microarray technology to diagnostic testing and functional studies of hearing impairment genes and the proteins they encode, will lead to improved classification and prediction of outcomes, as well as to rational and effective therapies for all forms of hearing impairment.

Glossary

ABR/EcochG: Auditory brainstem response and electrocochleography are companion evoked-potential studies that reveal the cochlear microphonic, summing potential, action potential and synchronous discharge of neural elements in the auditory pathway from cochlea to lateral lemniscus. The tests are used as tools to evaluate both auditory and neural integrity of the auditory system. While neither is a direct test of hearing, the results are powerful predictors of hearing status, when they are normal. In contrast, the absence of a synchronous ABR or EcochG does not always mean deafness is present.

Allele: One of several alternative forms of a gene or DNA sequence at a specific chromosomal location.

ATP (adenosine triphosphate): The principal immediate-energy source in all eukaryotic cells.

Autozygosity: Homozygosity for an allele that is identical by descent.

BAC (bacterial artificial chromosome): A vector designed for cloning relatively large DNA fragments between 50 and 200 kb. BACs are propagated in a host bacterial cell.

- Centromere:** Primary constriction of a chromosome, separating the short arm from the long arm. Its major function is to ensure correct segregation of homologous chromosomes during meiosis and mitosis.
- Codon:** Nucleotide triplet that specifies an amino acid, or a signal for terminating the synthesis of a polypeptide.
- Consensus sequence:** In genes or proteins, an idealized sequence in which each base or amino acid residue represents the one most frequently found at that position when many actual sequences are compared.
- Contig:** Continuous region of genomic DNA that has been cloned in a series of identifiable overlapping DNA clones.
- Degenerate code:** The genetic code is described as degenerate because more than one codon can encode the same amino acid.
- Dimerization:** The formation of a compound composed of two molecules.
- Dominant negative mutation:** The abnormal product of one allele disrupts the function of the product of the normal allele.
- Endonuclease:** An enzyme that can cut phosphodiester bonds that occur internally in a DNA chain.
- Epistasis:** Phenotypic expression is the result of interaction between alleles at different loci.
- EST (expressed sequence tag):** A sequence of part of the coding region of a gene.
- Eukaryote:** Each cell has a nucleus that contains the genetic material, surrounded by cytoplasm, which in turn is bounded by the plasma membrane that marks the periphery of the cell.
- Exon:** Segment of a gene that is decoded to give an mRNA product.
- Frameshift Mutation:** A mutation in which there is a deletion or insertion of a number of nucleotides that is not a multiple of three. This causes the codon reading frame to shift.
- Glutamic acid:** An amino acid that is part of the cochlear afferent cycle.
- Haploid gametes:** Cells having only a single copy of each chromosome.
- Haploinsufficiency:** A locus shows haploinsufficiency if producing a normal phenotype requires more gene product than the amount produced by a single copy.
- Haplotype:** The particular combination of alleles in a defined region of a single chromosome.
- Hardy-Weinberg (equilibrium) law:** The relationship between gene frequencies and genotype frequencies that is found in a population under certain conditions.
- Heteromultimeric proteins:** Proteins that consist of nonidentical subunits (coded by different genes).
- Heteroplasmy:** Two or more genetically distinct populations of mitochondria in a somatic cell tissue.
- Heterozygous:** The individual's genotype at the locus consists of two different alleles.
- Histones:** Proteins associated with DNA in the chromosomes, rich in basic amino acids (lysine or arginine) and virtually invariant throughout eukaryote evolution.
- Homeodomain:** Conserved DNA binding domain consisting of the 60 amino acids encoded by a homeobox gene.
- Homozygous:** The individual's genotype at the locus consists of two identical alleles.

Intron: Noncoding DNA that separates neighboring exons in a gene.

Linkage disequilibrium: Nonrandom association of alleles at linked loci.

Locus: The position of a gene or genetic marker on a chromosome.

Lod score: A measure of the likelihood of genetic linkage between loci. A score greater than +3 is often taken as evidence of linkage; one that is less than -2 is often taken as evidence against linkage.

Megakaryocytes: White blood cells that produce platelets by cytoplasmic budding.

Missense mutation: A nucleotide substitution that results in an amino acid change.

Multimeric complex: Structure composed of several identical or different subunits held together by weak bonds.

Nullisomy: A diploid cell missing both copies of the same chromosome.

Oligonucleotide: A short DNA molecule synthesized for use as a probe.

Open Reading Frame (ORF): A significantly long sequence of DNA in which there are no termination codons.

Penetrance: The probability that a given genotype will result in a particular phenotype.

Phenocopy: A phenotype that looks the same as one produced by a specific genotype, but has a different etiology.

Pleiotropy: Multiple phenotypic effects of a single gene.

Polycistronic mRNA: Includes coding regions representing more than one gene.

Polymorphism: The occurrence together in a population of two or more alleles at a locus, none of which are at a frequency that could be maintained by recurrent mutation alone.

Polyloid: Having multiple chromosome sets.

Positional cloning: Cloning of a gene which is dependent only on knowledge of its subchromosomal location.

Purine: A nitrogen-containing compound with a double-ring structure (e.g., adenine and guanine).

Pyrimidine: A nitrogen-containing compound with a single-ring structure (e.g., cytosine, thymine, and uracil).

Radiation hybrid: A type of somatic cell hybrid in which fragments of chromosomes of one cell type are generated by exposure to X-rays, and are subsequently allowed to integrate into the chromosomes of a second cell type.

Reproductive fitness: Relative reproductive success of a genotype as measured by survival, fecundity or other life history parameters.

Splice site: The boundary between an intron and exon. The introns are removed in the generation of mature mRNA.

Stop codon: One of the three codons (UAG, UAA and UGA) that terminate synthesis of a polypeptide.

Synteny: The property of occurring on the same chromosome.

Transcription: The assembly of a complementary single-stranded molecule of RNA on a DNA template.

Transfection: Transfer of a gene, or cDNA (next to a promoter), into a cell, enabling the transfected cell to form a new gene product.

Transition: A nucleotide substitution in which one purine is replaced by another, or one pyrimidine is replaced by another.

Translation: The assembly of a polypeptide chain from the coded information in the mRNA that directs the amino acid sequence of the chain.

Transversion: A nucleotide substitution of purine for pyrimidine, or vice versa.

VNTR (variable number of tandem repeats): A type of DNA polymorphism created by a tandem arrangement of multiple copies of short DNA sequences.

Wild type: Term used to indicate the normal allele or the normal phenotype.

YAC (yeast artificial chromosome): A linear cloning vector into which a large fragment of DNA can be inserted.

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Genes and Mutations in Hearing Impairment

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1. Introduction

In the last five years, scientists have made great advances in deciphering the genetic basis of hearing impairment. A fundamental knowledge of the structure of chromosomes and genes is required to appreciate the events leading to mutations causing hearing loss. This chapter covers the organization of chromosomes and genes, discusses the flow of information from DNA to RNA to protein, and delineates how different types of mutations lead to abnormal gene expression or gene products. The mutations described are a representative sampling, not an exhaustive list, of mutations in genes that lead to hearing loss. Many more examples are described in the literature and the list is growing monthly. The emphasis will be on mutations associated with human deafness (Griffith and Friedman, Chapter 6), although examples of mouse mutations will also be mentioned, because of the relevance of mouse models to human hearing loss (Steel, Chapter 8).

For additional background information regarding the structure of chromosomes and genes, the reader is referred to Lodish et al. (1995), Klug and Cummings (1997), and Lewis (1999).

2. Chromosome Structure

Eukaryotic cells contain large linear chromosomes, which carry the genetic material in the form of genes composed of DNA (Fig. 2.1). Most somatic cells contain two copies of each chromosome derived from the germ cells of each parent. Exceptions include: the haploid gametes, the sperm and egg cells, which contain only one set of chromosomes; the platelets and red blood cells, which lack a nucleus; and polyploid cells, such as liver regenerating cells and bone marrow megakaryocytes.

Chromosome size and number vary with species. In general, there is a correlation between the complexity of the species and the genome size. The yeast genome size is 14 megabases (Mb), whereas the genomes of the mouse

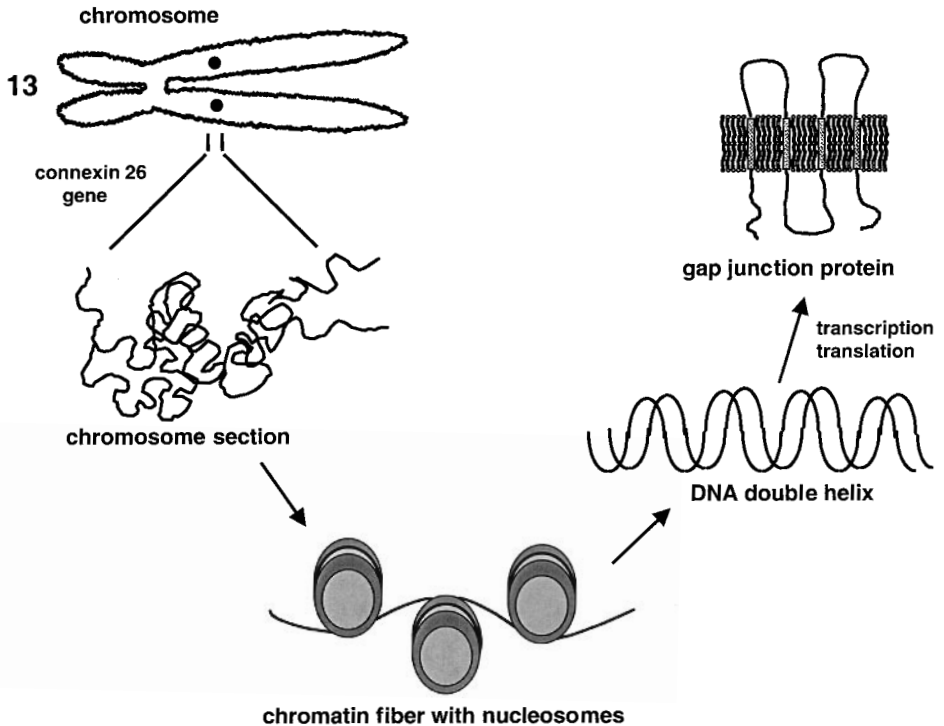


FIGURE 2.1. Individual genes lie next to one another (and in some cases, even overlap) on chromosomes. The ability of DNA to be compacted in the nucleus of each cell is a remarkable feat. DNA molecules wrap themselves around histone proteins to form chromatin, then further condensed to form the chromosomes. A nucleosome is composed of histones and 2 1/2 turns of DNA. When DNA is transcribed to RNA, it unwinds from the histone only in the particular site where transcription is occurring. The RNA is then translated into a functional protein. The gene that encodes the gap junction protein connexin 26 lies on human chromosome 13. Thirty to fifty percent of NSHL is due to mutations in the connexin 26 (*GJB2*) gene.

and human are identical in size at 3,000Mb. There are exceptions to this rule, however; the onion genome size is 15,000Mb.

Human cells have 23 sets of chromosomes. In the haploid human cell, 22 chromosomes are autosomal, or non-sex chromosomes, while the remaining one is an X or Y sex chromosome. The fusion of two haploid cells to form a diploid cell during fertilization enables one homologue from each pair of chromosomes to join to form paired chromosomes containing nearly identical DNA. The basic material of chromosomes is termed chromatin, and it is composed of DNA and its associated histone proteins.

The histone proteins serve to compact the DNA so that it may fit into a nucleus. An assemblage of eight histone proteins is encircled by a stretch

of 146 DNA nucleotides to form an 11 nm nucleosome (1 nm is a billionth of a meter). The nucleosomes, like beads on a string, further wind around each other to create the 30 nm chromatin fibers (Fig. 2.1). This compaction is necessary, since the human haploid genome, if stretched out, would reach the height of a person. A copy of this genome is present in each cell.

2.1 Chromosomal Abnormalities Leading to Deafness

Chromosome abnormalities can either be numerical or structural in nature, and usually lead to extreme phenotypes (Table 2.1). They often affect a large genomic DNA region, and can result in the loss or gain of partial or whole chromosomes. Large chromosome abnormalities can lead to embryonic lethality from the loss of one or more essential genes, or to a disease with several phenotypes, as is seen with syndromic hearing loss. The most common chromosome abnormalities are numerical and involve the loss or gain of whole chromosomes. Polyploidy, which describes the presence of an extra copy of the entire set of chromosomes, is not viable in humans. In contrast, some forms of aneuploidy (the loss or gain of one chromosome) are compatible with life (Giersch and Morton, Chapter 3). The most common form is the gain of an additional chromosome 21 (trisomy 21), which leads to Down syndrome. Some trisomy 21 patients exhibit conductive and/or sensorineural hearing loss, although the gene(s) contributing to the hearing loss are unknown (Gorlin et al. 1995).

Structural chromosomal abnormalities involve chromosome breaks, with rejoining of breakpoints in several possible configurations. The breaks may affect one or more genes, and lead to chromosomal translocations, inversions and deletions (Fig. 2.2A). When a translocation occurs, genetic material is transferred from one chromosome to another. An inversion is a reversal in the order of a chromosomal segment; genetic information is usually not lost, but the linear sequence of the genes is altered. A pericentric inversion involves the centromere, whereas a paracentric inversion does not include the centromere. Although chromosome breaks are uncommon in nonsyndromic sensorineural hearing loss (NSHL), a paracentric inversion is known to cause NSHL in the mutant deaf mouse, Snell's waltzer (Avraham et al. 1995). The chromosomal breakpoints of this inversion are near, but not within, the coding regions of the short ear (*Bmp5*) and Snell's

TABLE 2.1. Types of chromosomal mutations

Chromosomal Abnormalities	Consequence
Numerical	Aneuploidy Polyploidy
Structural	Translocations Deletions Inversions

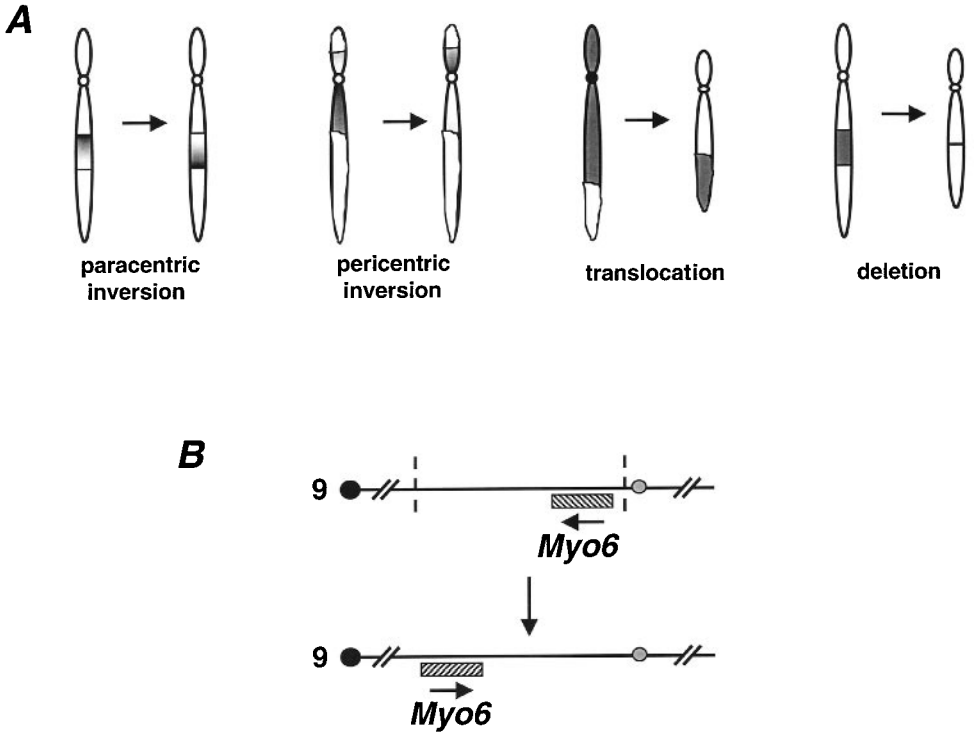


FIGURE 2.2. (A) Structural chromosomal abnormalities may lead to translocations, inversions, or deletions. (B) A paracentric inversion on mouse chromosome 9 leads to the *se^{sv}* combined phenotype of short ear (*se*) and Snell's waltzer (*sv*, deafness and circling) (Avraham et al. 1995). Breaks occur at the dotted lines, causing the myosin VI (*Myo6*) gene, normally transcribed in the direction shown by the arrow, to be inverted. Upstream regulatory regions, shown in grey, are lost, leading to down-regulation of myosin VI expression.

waltzer (*Myo6*) genes (Fig. 2.2B). No other genes appear to be affected, since the inverted DNA remains intact (except for small deletions at each breakpoint) and the mice only harbor phenotypes representative of the two genes. The breakpoints near the *Bmp5* and *Myo6* genes affect the downstream and upstream regions of these genes, respectively, leading to skeletal (for *Bmp5*; DiLeone 1998) and hearing (for *Myo6*) abnormalities in these mice. The consequence of this inversion is a position effect, where the expression of a gene is altered due to the relocation of chromosomal regions.

Most chromosomal deletions causing NSHL are intragenic (within the gene) and comprise no more than a few base pairs. One exception is the X-linked *DFN3* locus, with mutations in the *POU3F4* gene. Several chromo-

somal deletions encompassing this gene have been reported, ranging from 250kb to several megabases (Huber et al. 1994). This region of the X chromosome appears to be gene-poor because hearing loss (resulting from deletion of the *POU3F4* gene) is most often the only phenotype.

3. From DNA to Protein: Transcription and Translation

The flow of information begins with the genetic material, the DNA. All cells contain the same genetic material in the form of DNA, but the levels at which this material is expressed, in the form of protein, varies between each tissue. The process of “expression” is a complex one, involving many steps, all of which may be affected by genetic mutations (Fig. 2.3). The first step, DNA transcription, changes the DNA information into RNA. There are three major types of RNA molecules produced by transcription: messenger RNA (mRNA), transfer RNA (tRNA) and ribosomal RNA (rRNA). mRNAs are utilized in the process of translation and are the template for protein synthesis. rRNAs serve as structural components of the translational machinery, whereas tRNAs serve as the carriers that bring in new amino acids for the growing peptide chain.

DNA is transcribed into mRNA in the nucleus. Sequences in the DNA, termed promoters, are recognized by transcription factors that recruit the RNA synthesis machinery to the gene and initiate RNA synthesis at that site. After RNA synthesis is initiated, the growing RNA is elongated until a termination signal is reached and the RNA synthesis machinery is

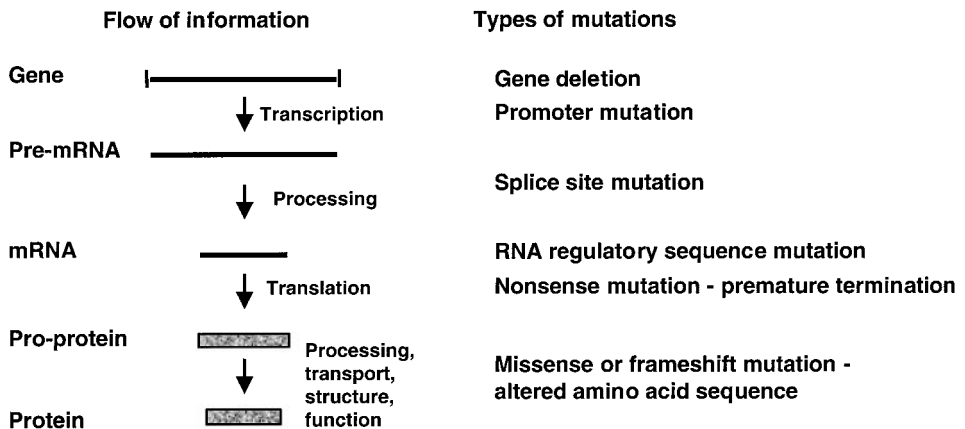


FIGURE 2.3. A schematic view of the flow of information within a cell, beginning with DNA as the genetic material and ending with protein as the “expression” of the genetic material. Mutations can occur at each of these steps. Modified with permission from Pearson Education Limited (Carter and Murphey 1999).

released from the DNA. The initial mRNA product, termed the pre-mRNA, contains both exons, regions that encode for proteins, and introns, intervening sequences that do not encode protein. In an essential process called mRNA splicing, the noncoding introns are removed out of the pre-mRNA to form the mature and functional mRNA (Fig. 2.4).

After the mRNAs are fully processed in the nucleus, they are transported through the nuclear pores into the cytoplasm. Here the mRNAs are assembled onto ribosomes, the translation machinery that converts the information encoded in each mRNA into protein. Each ribosome consists of two major subunits, one large and one small, and these subunits are made up of both protein and ribosomal RNA (rRNA) components. The process of translation, like that of transcription, can be divided into three steps: initiation, elongation and termination. All three of these processes are catalyzed by the ribosomal proteins, but are dependent on proper rRNA function.

Translation initiation begins with the binding of the small ribosomal subunit to a recognition sequence on the mRNA (Fig. 2.5). All 20 amino acids are encoded by three base sequences of mRNA termed codons. The translational recognition sequence includes the sequences surrounding the “start codon,” usually an AUG codon encoding the amino acid methionine. After binding to the recognition sequence, a methionine-charged tRNA is brought into place, the large subunit of the ribosome joins the complex, and translation is initiated. Elongation of the peptide occurs catalytically with the ribosome moving along the mRNA in the 5′ to 3′ direction. The open reading frame refers to the subset of nucleotides of the mRNA that are used as the template to create protein. The ribosome shifts to the next three bases in the sequence (the next codon) and adds the appropriate amino acid to the growing peptide. The process of translation is halted when a stop codon—UAA, UAG, or UGA—is encountered.

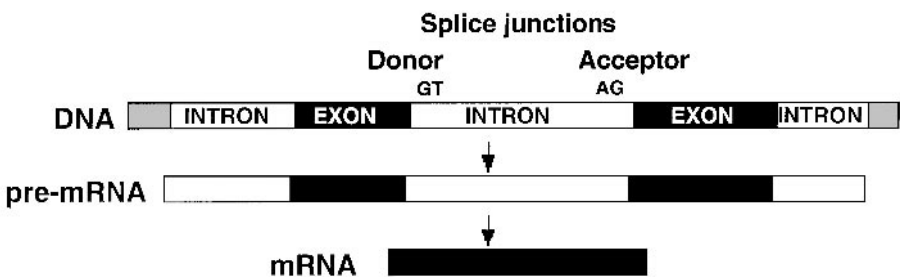


FIGURE 2.4. The coding sequence of genes are composed of exons that are separated by intervening sequences, introns. The full length of the gene is transcribed into a primary RNA transcript that undergoes RNA splicing. Introns are removed, allowing the RNA segments to be joined at the splice junctions to form mRNA.

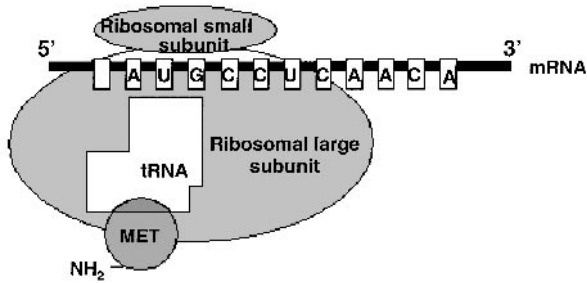


FIGURE 2.5. Translation of the mRNA begins with the formation of an initiation complex. The first step in the formation of the initiation complex is the binding of the small ribosomal subunit to a recognition sequence on the mRNA. The translational recognition sequence includes the sequences surrounding the “start codon,” usually an AUG codon encoding the amino acid methionine. After binding to the recognition sequence, a methionine-charged tRNA is brought into place, the large subunit of the ribosome joins the complex, and translation is initiated. Elongation of the peptide occurs catalytically with the ribosome moving along the mRNA in the 5' to 3' direction.

4. Mutations

Mutations are defined as changes in the chemical composition of DNA and can manifest themselves either during transcription or translation. The end result, in either case, is altered expression of the protein. Mutations can occur in somatic cells, in which case they only affect the individual in whose cells the mutation is present. Mutations in the germ line (sperm or ovum), however, can be passed on to subsequent generations.

4.1 Mutations at the Level of the Gene

Mutations at the level of DNA can ultimately result in damage at the level of the protein. There are three major types of DNA mutations: point mutations, insertions and deletions (Fig. 2.6; Table 2.2). A point mutation, or substitution, is a change in a single nucleotide. Point mutations may be due to transitions or transversions of the nucleotide. A transition occurs when a purine replaces a purine (A to G or G to A), or a pyrimidine replaces a pyrimidine (C to T or T to C). In a transversion, a purine replaces a pyrimidine and vice versa (A or G to T or C).

As each amino acid is encoded by a three-nucleotide codon, single nucleotide changes in the DNA often lead to an amino acid change. This change from one amino acid to another is termed a missense mutation. When a missense mutation occurs, the structure and function of the protein can be affected. In some cases, the change in the DNA sequence does not alter the encoded amino acid. This change is termed a polymorphism or a

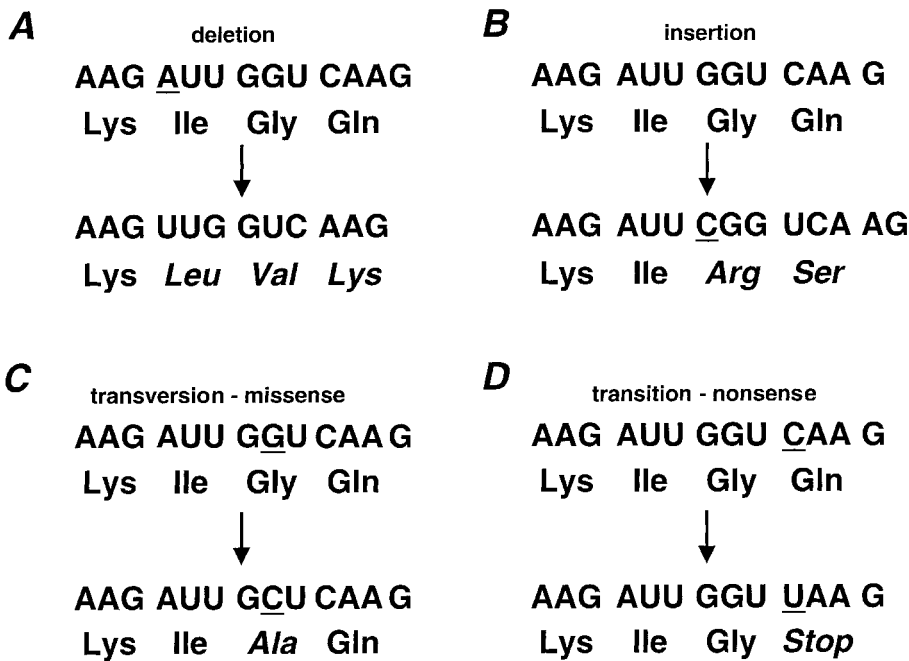


FIGURE 2.6. Mutations may be in the form of deletions, insertions or substitutions. In (A) and (B), a deletion or insertion of one nucleotide forms a frameshift, changing the subsequent amino acids. In (C), a transversion leads to a missense mutation, affecting only one amino acid. In (D), a transition leads to a nonsense mutation, forming a stop codon.

TABLE 2.2. Types of DNA mutations

DNA mutation	Examples of resulting mutation
Point (substitution, transversion or transition)	Missense—altered amino acid Nonsense—stop codon
Deletion/ insertion	
Multiple of 3	Deletion/insertion of amino acid(s)
Not a multiple of 3	Frameshift, truncation

variant and may occur naturally in the population, rather than be the cause of a disease. Finally, when a DNA mutation changes the amino-acid codon to a stop codon (UAA, UAG, or UGA in RNA), a nonsense mutation occurs. This mutation can lead to protein truncation and can clearly affect protein function.

Insertions and deletions of DNA nucleotides also change the encoded protein. The addition or deletion of three nucleotides will add or delete an amino acid, which may have dire consequences for the protein. Alterna-

tively, if the addition or deletion is not in multiples of three, a frameshift occurs, since the codon reading frame is now altered. Frameshifts result in the addition of incorrect amino acids to the elongating protein. Ultimately, frameshifts will terminate at a stop codon farther downstream. Because of the addition of incorrect amino acids, in some cases, the mutated protein is recognized by the cell as nonfunctional. This may be because the protein is not targeted to its correct location within the cell, or because the protein is not exhibiting the correct enzymatic activity. In these cases, the mutated protein is often destabilized and is degraded by the cell.

In addition to DNA mutations that directly alter protein sequence, point mutations, insertions and deletions can also affect sequences essential for the initiation of transcription and translation. Ultimately, these types of mutations have the same result as mutations in the coding regions, namely that the encoded protein is not correctly expressed.

Silent mutations, or variants, occur when a substitution in a nucleotide occurs without altering the amino acid. Single-nucleotide polymorphisms (SNPs), the most frequent type of variation in the human genome, may explain susceptibility to common genetic traits and diseases and are currently being identified on a large scale as part of the Human Genome Project (Wang et al. 1998a).

4.1.1 Mutations in the Coding DNA Sequence Affect the Translation of RNA into Protein and Cause Hearing Loss

There are numerous examples of missense, nonsense, deletion, insertion, and frameshift mutations in NSHL (Table 2.3), all of which affect translation of RNA into protein. One example of a locus that has multiple missense mutations that affect protein translation is the *DFNA2* region. *DFNA2* contains at least two deafness genes, *KCNQ4*, which codes for a potassium channel (Kubisch et al. 1999), and *GJB3*, which codes for a gap junction protein (Xia et al. 1998). One French family, exhibiting dominant profound hearing loss, was found to harbor a single copy of a missense mutation in *KCNQ4*. In this family, although the remainder of the protein is translated properly, a single glycine at amino acid 285 is changed to a serine. This glycine residue is normally located in the potassium channel pore, a highly conserved region of the protein. Replacement of this glycine with a serine results in a dysfunctional potassium channel. Three additional missense mutations have been found in *KCNQ4* in Dutch and American *DFNA2* families, and in all cases, the missense mutations alter conserved and presumably essential regions in the protein (Coucke et al. 1999).

For two dominant loci, *DFNA8* and *DFNA12*, missense mutations in *TECTA* lead to dominant NSHL (Verhoeven et al. 1998). Interestingly, in one Belgian family, there are two missense mutations 12 bases apart. The mutations may interact, or only one may contribute to the disease, with the second mutation being a rare polymorphism, since it was not detected in 40

TABLE 2.3. Examples of mutations in the DNA sequence in NSHL

Gene	Locus	Type of mutation	Nucleotide change	Mutation	Reference
<i>KCNQ4</i> <i>GJB3</i>	<i>DFA2</i>	Deletion/frameshift	13bp	211del13 ^a	Coucke et al. 1999
	<i>DFA2</i>	Transition/missense	Gag→Aag ^b	Q183K ^c	Xia et al. 1998
	<i>DFA2</i>	Transition/nonsense	Cga→Tga	R180X ^d	Xia et al. 1998
<i>TECTA</i>	<i>DFA8</i>	Transition/missense	tAt→tGt	Y1870C	Verhoeven et al. 1998
	<i>DFA12</i>	Transition/missense	Ctc→Ttc	L1820F	Verhoeven et al. 1998
		Transition/missense	gGt→gAt	G1824D	
<i>COCH</i>	<i>DFA12</i>	Transversion/missense	tGc→tCc	C1619S	Alloisio et al. 1999
	<i>DFNB12</i>	Transition/donor splice site	G→A	Truncation/stop	Mustapha et al. 1999
	<i>DFA9</i>	Transversion/missense	gTla→gGa	V66G	Robertson et al. 1998
	<i>DFA9</i>	Transition/missense	gGa→gAa	G88E	Robertson et al. 1998
	<i>DFA9</i>	Transition/missense	Tgg→Cgg	W117R	Robertson et al. 1998
	<i>DFA9</i>	Transition/missense	Cca→Tca	P51S	de Kok et al. 1999
<i>OTOF</i>	<i>DFNB9</i>	Transversion/nonsense	aTg→aAg	Y730X	Yasunaga et al. 1999
<i>MYO15</i>	<i>DFNB3</i>	Transversion/missense	Atc→Ttc	I892F	Wang et al. 1998b
		Transversion/missense	Aac→Tac	N890Y	Wang et al. 1998b
		Transversion/nonsense	Aaa→Taa	K1300X	Wang et al. 1998b

^a Nomenclature for deletion (i.e., 211del13 is a 13-bp deletion at nucleotide 211).

^b Nucleotides in lower case remain the same; nucleotide in upper case is mutated.

^c Nomenclature for conversion of one amino acid to another (i.e. Q183K is glutamine to lysine at amino acid number 183 of the protein).

^d X = stop codon.

unaffected family members, or 100 non-related individuals. In all cases, the conserved zona pellucida domain of the α -tectorin protein is compromised by the replacement of alternate amino acids, suggesting this region has a crucial function for this protein (Verhoeven et al. 1998).

Connexin 26 is a transmembrane protein that forms cylindrical channels in gap junctions used to transfer small molecules between cells. This gap junction protein is highly expressed between supporting cells of the inner ear, and in the spiral lamina facing the endolymphatic duct and may play a role in K^+ recycling in the inner ear (Spicer and Schulte 1996). All connexin 26 mutations characterized to date are either nucleotide substitutions (missense/nonsense/splicing mutations), or insertions and deletions ranging from small (1 bp) to large (38 bp) in the coding region (mutations updated regularly in the Connexin 26 (*GJB2*) Deafness Homepage (World Wide Web URL: <http://www.iro.es/cx26deaf.html>). The common 35delG mutation is a one-base-pair deletion in a stretch of six G nucleotides in codon 10 that results in a frameshift; a glycine is converted to a valine at codon 12 and a stop codon is formed at codon 13 (Zelante et al. 1997). In other families, an insertion has been detected at the same site, 35insG, leading to a frameshift and stop codon at codon 47 (Estivill et al. 1998). In both cases, it is highly unlikely that any protein is translated. A mutation involving both an insertion and deletion has been identified in a family with recessive NSHL (Sobe et al. 2000). A deletion of 12 bp occurred, with the insertion of one nucleotide, to form a 51del12insA mutation. This mutation occurs in the first intracellular amino terminus domain, leading to a stop codon at the beginning of the first extracellular domain. A frameshift is formed in the amino-terminal portion of the protein, resulting in 26 additional novel amino acids followed by a premature termination.

Additional examples of mutations in the DNA sequence affecting the translation of RNA into protein and causing NSHL are shown in Table 2.3.

4.1.2 Mutations in Components of the Translation Machinery Cause Hearing Loss

The RNA components of the ribosome are essential for translation of mRNA into protein. Hearing loss has been correlated with mutations in both tRNA genes and the 12S rRNA gene, genes found on the mitochondrial genome (Fischel-Ghodsian, Chapter 7). Unlike the deafness genes described elsewhere in this chapter, this mitochondrial-dependent hearing loss is transmitted directly from mother to child. Presumably, these mutations alter the translational machinery in the inner ear, and compromise the production of essential protein components of the mitochondria.

4.2 Mutations that Affect the Flow of Information from DNA to RNA

The process of hearing, as well as development of the hearing organ, occurs as the result of a programmed expression of genes. As a result, the tissues of the ear are sensitive to alterations in all aspects of the process of transcription of DNA into mRNA. Mutations have been found in transcription factors that program ear development. In addition, deafness results from mutations that alter the mRNA expression of individual genes essential for hearing. These mutations include alterations in the gene promoters and in the mRNA splicing sites. Without a functional promoter, genes are not properly transcribed. As a result of promoter mutations, a gene may be expressed at a lower level than normal or expression in critical tissues may simply be lost. The lack of sufficient transcript often results in a decrease in final protein levels. This lack of protein may, in turn, result in deafness if this protein is critical for ear development, or for neurosensory function. In addition, mutations have been observed that allow for gene transcription, but result in mRNA degradation such that, in the end, no protein is produced.

4.2.1 Transcription Factor Mutations Lead to Hearing Loss

Mutations in transcription factors have been found to be the basis for both syndromic and nonsyndromic deafness. Transcription factors are a group of proteins that bind DNA and initiate transcription at specific sites on the DNA (Fig. 2.7). Eukaryotic genes require several types of transcription factors in order to be transcribed. The genes for these transcription factors may be close to the genes they regulate, or far away. They recognize the

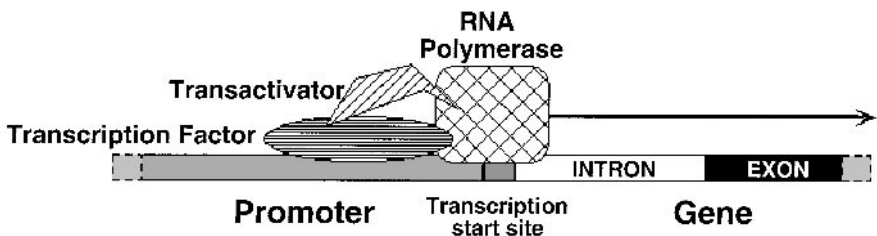


FIGURE 2.7. Initiation of RNA transcription. To initiate gene transcription, the promoter of the gene is bound by a series of transcription factors that recognize specific sequences in the promoter DNA. Transactivators then join the complex by binding to specific sites on the transcription factors. This assemblage of transcriptional regulators recruits RNA polymerase, and RNA transcription is begun.

promoter of these genes, a region of DNA found upstream of the coding sequences. These transcription factors, in some cases, are required for proper bone development. In other cases, the transcription factors are required for sensory-cell differentiation at later stages of development. Surprisingly, a wide variety of different transcription factor types have been found to encode deafness genes. Thus far, the downstream genes activated by these transcription factors have not been identified, but clearly these genes are critical for ear development. In this section, a review of some of the transcription factors known to be required for hearing, and the phenotypes associated with these transcription mutations, is presented.

In humans, a mutation in the *POU4F3* transcription factor gene (also called *Brn3c* and *Brn-3.1*) is the basis for progressive, nonsyndromic dominant hearing loss (*DFNA15*) (Vahava et al. 1998). This transcription factor, which is specifically expressed in the cochlea, has a conserved DNA binding domain, termed a homeodomain, found in a wide variety of transcription factors that have been shown to be important for embryonic development in all species (Semenza 1998). The mutation in *POU4F3* is an 8-bp deletion within the homeodomain. This deletion results in a frameshift that produces a protein that is truncated 40 amino acids prematurely. This truncated *POU4F3* protein acts in a dominant fashion to disrupt gene transcription in the cochlea.

Targeted deletion of the mouse *Pou4f3* gene also leads to profound deafness, confirming the general importance of this transcription factor in mammalian inner ear development (Erkman et al. 1996). In the *Pou4f3* mutant mice, the sensory cells of the inner ear, as well as their associated nerves, are not present, although the shape and development of the inner ear is normal. It is the loss of these cell types that results in deafness, and *Pou4f3* protein is clearly required for terminal differentiation of these cell types.

Mutations in a related transcription factor, *POU3F4*, are responsible for X-linked mixed hearing loss, characterized by stapes fixation and progressive sensorineural deafness (*DFN3*) (de Kok et al. 1995). This homeodomain transcription factor has been implicated in bone development, since patients with *POU3F4* mutations have a fixed footplate of the stapes and deficient or absent bone between the lateral end of the meatus and the basal turn of the cochlea. Patients also exhibit cochlear defects, suggesting that *POU3F4* is involved in multiple stages of ear development.

POU-type transcription factors are not the only inner ear specific transcriptional regulators. In the mouse, a transcriptional regulator of the forkhead family, *Foxi1* has been found to encode a nonsyndromic deafness gene (Hulander et al. 1998). *Foxi1* mutant mice have profound defects in the structure of the bones of the inner ear, and, as a result, the sensory regions of the cochlea and vestibular apparatus do not develop. The result is a "common cavity," a malformation seen in a portion of human congenital

inner ear defects. Thus far, no human patients have been identified with alterations in *Foxi1*, but it is considered a good candidate for a human deafness gene.

Gene-targeted mutagenesis of *Math1*, the mouse homologue of the *Drosophila* transcription factor *atonal*, has been found to cause deafness in mice (Bermingham et al. 1999). Most significantly, the hair cells in these mice never form, indicating that this transcription factor regulates the genesis of hair cells.

Mutations in transcription factors can also result in syndromic deafness. A summary of these transcription factors and their associated syndrome genes is shown in Table 2.4. Of these diseases, the best characterized is Waardenburg syndrome (WS). Waardenburg patients have an auditory-pigmentary syndrome characteristic of a defect in melanocyte development (reviewed in Read and Newton 1997). Most patients have mutations in the transcription factor *PAX3* and, depending on the penetrance of the mutation, patients can exhibit dystopia canthorum (lateral displacement of eyes; WS type 1) or musculoskeletal abnormalities (WS type 3), in addition to sensorineural deafness and depigmentation. Patients with deletions of the *PAX3* gene have phenotypes indistinguishable from patients that have small substitutions in the DNA-binding domains of *PAX3*, and it has been observed that two very similar mutations can result in different phenotypes in different families. To account for these data, it has been proposed that gene dosage may play an important role in the cause of WS, with patients having a lower effective dose of *PAX3* exhibiting more severe phenotypes.

Waardenburg patients with a milder series of melanocyte defects have mutations in the transcription factor *MITF* (Tassabehji et al. 1994). *MITF* has been shown to transcriptionally transactivate the gene for tyrosinase, a key enzyme in melanogenesis and overexpression of *MITF* can convert fibroblasts into cells with melanocyte characteristics (Tachibana et al. 1996). Defects in *MITF* likely lead to anomalies during melanocyte differentiation which cause the hearing defects and hypopigmentation seen in WS type 2 patients.

Only recently has the role of *MITF* in melanocyte differentiation been integrated into the phenotypes seen in patients with *PAX3* mutations. *PAX3* can transcriptionally transactivate the *MITF* promoter (Watanabe et al. 1998). These results suggest that *PAX3* directly regulates *MITF*, and that *MITF*, as a gene that requires functional *PAX3* for expression, is responsible for the defects seen in melanocyte differentiation in WS types 1 and 3. These studies provide the first example of the cascade of transcription factors required for inner ear development.

So far, over eight transcription factors, each a member of a distinct transcription-factor family, have been identified as key players in the regulation of genes required for inner ear development. In most cases, the genes regulated by the transcription factors have not been precisely identified. Ultimately, these downstream genes are required for both the development

TABLE 2.4. Transcription factor genes responsible for SHL

Syndrome	Dom/ Rec	Affected tissues	Transcription Factor Gene (homologue)	Transcription Factor Protein type	Reference
Waardenburg syndrome type 1	Dom	Inner ear; skin, hair & eye pigmentation; lateral displacement of eyes	<i>PAX3</i> (Mouse <i>splotch</i>)	PAX family with paired domain & homeodomain	Read and Newton 1997
Waardenburg syndrome type 2	Dom	Inner ear; skin, hair & eye pigmentation	<i>MITF</i> (Mouse <i>microphthalmia</i>)	Basic helix-loop-helix leucine zipper	Read and Newton 1997
Waardenburg syndrome type 3	Dom	Inner ear; skin, hair & eye pigmentation; limb anomalies	<i>PAX3</i> (Mouse <i>splotch</i>)	PAX family with paired domain & homeodomain	Read and Newton 1997
Brachio-Oto-Renal (BOR) syndrome	Dom	Inner ear, Kidney	<i>EYAI</i> (<i>Drosophila</i> eyes <i>absent</i>)	Transcriptional co-activator	Abdelhak et al. 1997
Townes-Brocks syndrome (TBS)	Dom	Ear, anal, renal, and limb anomalies	<i>SALL1</i> (<i>Drosophila spalt</i>)	Zinc finger transcription factor	Kohlhase et al. 1998

Dom = dominant; Rec = recessive.

of the bones in the outer, middle and inner ear, as well as the differentiation of the nerves and sensory regions found in the inner ear.

4.2.2 Mutations that Alter Splicing Patterns Lead to Inner Ear Defects

The processing of primary RNA transcripts to remove intronic sequences is essential. For proper splicing to occur, small nuclear ribonucleoproteins recognize short specific sequences at the junctions between introns and exons (usually GT at the 5' end of the intron and AG at the 3' end of the intron) (Fig. 2.4). It has been found that mutations that alter these consensus sequences alter splicing patterns. Splice-site mutations can result in exon skipping, whereby the resulting mRNA is missing a coding exon. Splice-site mutations can also lead to the inclusion of intronic sequences within the mRNA. In both cases, the resulting mRNA is often nonfunctional. The incorrectly spliced mRNA may encode a protein with an internal deletion as a result of exon skipping, in frame. Alternatively, and more commonly, the incorrectly spliced mRNA can result in the addition of improper amino acid sequences to the protein, as well as termination of translation resulting in a truncated protein product.

Mutations that affect both the 5' donor and 3' acceptor splice sites, as well as mutations that affect intermediates in the splicing process, have been observed in both syndromic and nonsyndromic deafness genes. An example of an array of splice site mutations found in one gene, myosin VIIa (*MYO7A*), is shown in Table 2.5. Interestingly, in this case, splice-site mutations can yield different phenotypes: in some cases the mutations result in Usher syndrome type 1B, a syndromic form of deafness with retinitis pigmentosa, whereas in other cases the mutations lead to nonsyndromic deafness (*DFNB2*). Clearly, different tissues have different sensitivities to alterations at splice acceptor and donor sites.

Splicing defects can also be responsible for dominant deafness. The dominantly inherited progressive hearing loss (*DFNA1*) seen in a large kindred from Costa Rica is due to a transversion in the splice donor of an exon of the diaphanous gene (Lynch et al. 1997). The observed G to T transversion disrupts the consensus AG sequence at the 5' splice donor of the next to last exon. A shift in the open reading frame results and 21 novel amino acids are added to the protein before the peptide is truncated prematurely. This truncated protein acts in a dominant fashion to disrupt hearing. Diaphanous is a member of the formin family of proteins and may have a function in the cell cytoskeleton.

4.2.3 Deafness Mutations May Result in mRNA Destabilization

One last way that gene transcription can be altered by mutation is by the destabilization of the mRNA itself. Even small mutations can produce mRNAs that are degraded in the cell, resulting in a phenotype that is equivalent to a total gene deletion. One such example is seen for the mouse

TABLE 2.5. Splice-site mutations in the gene encoding human myosin VIIA

Exon	Alteration ^a	Sequence ^b	Mutation type	Phenotype—Disease	Reference
3	-2nt a→g	ccatagG→ccatggG	3' acceptor site	<i>DFNB2</i> —nonsyndromic deafness	Liu et al. 1997
5	+1nt g→a	AGgtg→AGgtg	5' donor site	<i>USH1B</i> —Usher syndrome	Adato et al. 1997
14	-8nt c→g	ctccccagG→gtccccagG	3' acceptor site	<i>USH1B</i> —Usher syndrome	Weston et al. 1996
16	1797 g→a	TGgtg→TAgtg	5' donor site	<i>DFNB2</i> —nonsyndromic deafness	Weil et al. 1997
18	+1nt g→a	AGgtg→AGgtg	5' donor site	<i>USH1B</i> —Usher syndrome	Adato et al. 1997
24	-21nt g→a		Splicing intermediate	<i>USH1B</i> —Usher syndrome	Janecke et al. 1999
29	+2nt t→a	AGgtt→AGgat	5' donor site	<i>USH1B</i> —Usher syndrome	Levy et al. 1997

^a Location of mutation relative to the exon (in the first column), and the precise mutation that is found. (i.e., in the first example, -2nt means two nucleotides before exon 3 and the change is from "a" to "g"). All these mutations are found in the noncoding introns except in one case, where the alteration is in the coding sequence (1797 g→a).

^b Sequence in the region of the mutation. Intron sequence is shown in lowercase and exon sequence in uppercase. The italicized nucleotide is the nucleotide altered in the mutation. Underlined are the 5' donor splice site consensus "ag" and the 3' acceptor splice site consensus "gt."

mutant deafwaddler (*dfw*). In the *dfw*^{2J} allele, a two-base-pair deletion is present (Street et al. 1998). This mutation would be predicted to result in a frame shift and a truncated protein. Northern analysis, however, confirmed that this small deletion resulted in the destabilization of the mRNA. The mechanism whereby mRNAs that encode nonfunctional or truncated proteins are recognized and degraded by the cell has not been elucidated.

5. Summary

The programmed expression of proteins is required for hearing. This chapter describes the structure of chromosomes and genes and delineates how the processes of DNA transcription and RNA translation are required to form functional proteins. In cases of genetic hearing loss, mutations have occurred in either transcription or translation of the genes that encode for proteins crucial for the proper development, structure and function of the inner ear. By identifying these genes, and defining the mutation in them that cause deafness, a better understanding of the biology of hearing will be attained. These studies may one day in the future enable the "correction" of genetic mutations through gene therapy, or regeneration of hair cells. New sensory hair cells are not formed postnatally, and during aging these important cells degenerate. Studies into why and how the inner ear cells degenerate during "accelerated" hearing loss will likely provide clues to what happens during the normal aging process.

Acknowledgments. The authors are supported by grants from the NIH/Fogarty International Center Grant 1 R03 TW01108-01 and the European Commission (QLG2-CT-1999-00988) (K.B.A.), and the Deafness Research Foundation (T.H.).

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3

Mapping and Cloning of Genes for Inherited Hearing Impairment

ROBERT F. MUELLER, GUY VAN CAMP and NICK J. LENCH

1. Introduction

The last few years have seen rapid progress in the mapping and cloning of genes for syndromic and nonsyndromic inherited hearing impairment. Gene cloning can involve a number of different approaches, which include functional, candidate, positional, and positional candidate cloning.

Functional cloning was the first method used to clone human genes. It involves studying cells or tissues affected by the disorder, identifying an altered protein or transcript specific to that tissue, and using this information to design a DNA probe to identify and isolate the gene responsible. The structural complexity of the inner ear, along with the large number of different proteins expressed within it, has seriously limited the use of this approach in the identification of genes for inherited hearing impairment.

The candidate gene approach involves screening genes that have been characterized and, because of knowledge about their function (or likely function) and/or pattern of expression, are thought to be responsible for the disorder even in the absence of information on their location in the genome. This approach was used in the identification of one of the genes responsible for the syndromic form of inherited hearing impairment known as Alport syndrome. Alport syndrome is characterized by the combination of glomerulonephritis and progressive high-frequency sensorineural hearing impairment. Both the renal involvement and hearing impairment usually manifest in adolescence or early adult life. Alport syndrome is genetically heterogeneous with autosomal dominant, recessive, and X-linked patterns of inheritance reported, the latter being the most common form. Immunological studies of renal biopsies from individuals with Alport syndrome showed renal glomerular basement membrane abnormalities of the third (*COL4A3*), fourth (*COL4A4*) and fifth (*COL4A5*) alpha chains of type IV collagen (Kashtan et al. 1986; Kleppel et al. 1987). The *COL4A5* gene mapped to the X chromosome (Vetrie et al. 1992) suggested it as a likely candidate gene which was confirmed by identification of mutations

in a number of individuals with Alport syndrome (Tryggvason et al. 1993). Perhaps not surprisingly, mutations have also been detected in the *COL4A3* and *COL4A4* genes on chromosome 2 in persons with the autosomal-recessive forms of Alport syndrome (Boye et al. 1998).

Because of the limited knowledge of the detailed structure and function of the inner ear, the candidate gene approach has found limited use in the identification of genes responsible for inherited hearing impairment, especially in the case of genes for nonsyndromic inherited hearing impairment. As in most other areas of inherited human diseases, the positional candidate approach has found widespread use in the identification of genes for inherited hearing impairment.

2. Positional Cloning

Positional cloning involves identifying transcripts in the interval of the region of the chromosome to which a gene has been mapped and screening those transcripts for mutations. Therefore, the first step in positional cloning is the mapping of the gene responsible through linkage analysis in family studies.

2.1 *Linkage and Linkage Analysis*

Mendel's third law, the principle of independent assortment, states that members of different gene pairs assort to gametes independently of one another. While this is true of genes on different chromosomes, it will not always be true for genes that are on the same chromosome. An exchange of genetic material, or what is known as crossing-over or recombination, occurs on average two to three times in each meiosis between homologous chromosomes. However, if two loci are positioned sufficiently close together on the same chromosome, recombination between them will be a rare event (Fig. 3.1). If the alleles at two loci are inherited together more often than would occur by chance, then they are said to be linked. Linkage analysis involves studying the pattern of segregation of polymorphic DNA markers located throughout the chromosomes in families in which a disorder is segregating.

2.2 *Polymorphic DNA Markers*

Variation in the nucleotide sequence, or what are called DNA sequence variants, of the human genome is common. This variation is inherited in a Mendelian codominant manner and usually occurs in intergenic noncoding DNA, and therefore has no phenotypic consequences. There are different

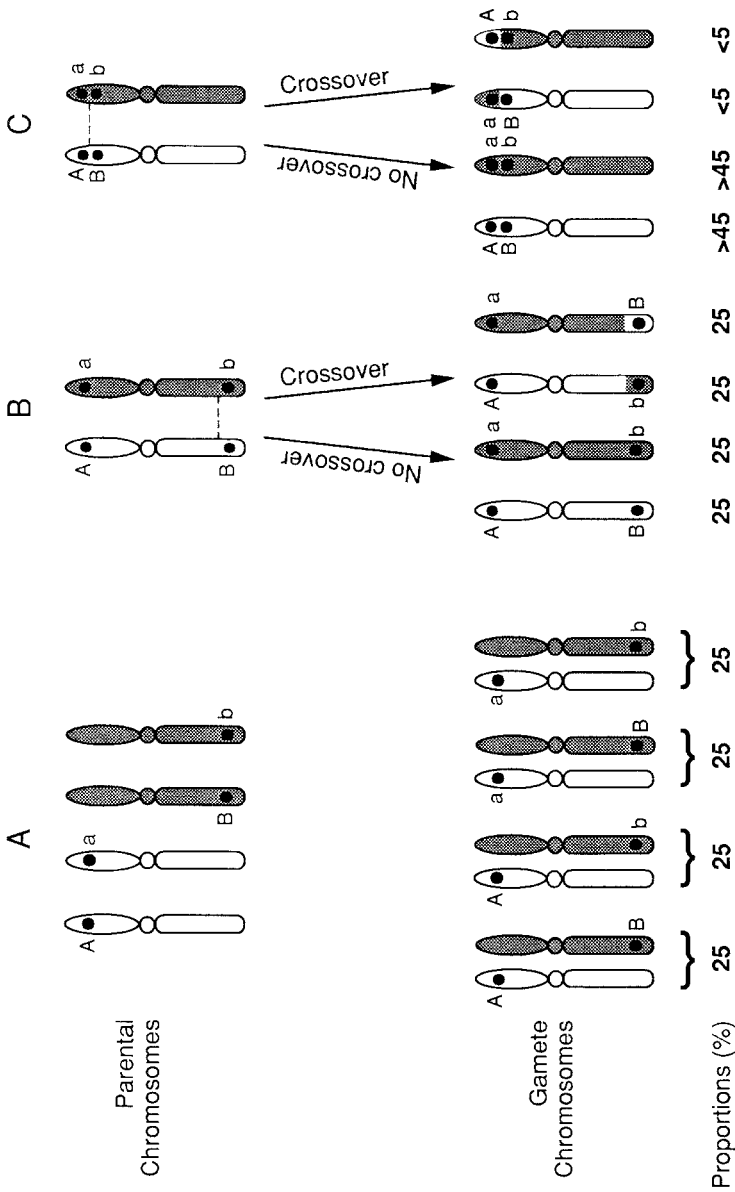


FIGURE 3.1. Segregation at meiosis of alleles at two loci. In (A) the loci are on different chromosomes and segregate independently. In (B), although on the same chromosome, they assort independently because they are widely separated. In (C) they are closely adjacent so that a crossover is unlikely, i.e., they are linked. (Reprinted from Emery's Elements of Medical Genetics, 10th ed, Mueller, RF, Fig. 3.1, Copyright 1998, by permission of Churchill Livingstone.)

types of DNA sequence variants that can be used in linkage analysis. The oldest, restriction fragment length polymorphisms (RFLPs), owing to their limited variation, have almost exclusively been replaced by a subset of variable number tandem repeats (VNTRs) known as microsatellites. The latter are likely to be succeeded in the near future by single nucleotide polymorphisms (SNPs).

VNTR polymorphisms are due to the presence of a different number of tandem repeats of short DNA sequences including either di-, tri- or tetranucleotide repeats known as short tandem repeats (STRs). The most commonly used VNTRs are dinucleotide repeats which occur some 50,000 to 100,000 times in the genome and consist of blocks of variable numbers of tandem repeats of the dinucleotide CA:GT constituting so-called CA repeats or microsatellites (Weber and May 1989). Microsatellites are highly polymorphic and some 8,000 or more have been identified (Weissenbach et al. 1992; Gyapay et al. 1994; Dib et al. 1996) and mapped to the human genome providing a skeleton framework of the human genome for linkage analysis.

More recently, a third generation of polymorphic DNA variants based on single nucleotide polymorphisms (SNPs) have been identified (Wang et al. 1998b). Some 700 to 900 SNPs at appropriate intervals throughout the genome will allow the possibility of mapping a disorder in a single analysis by DNA chip technology (Kruglyak 1997).

2.3 *Recombination Fraction*

The recombination fraction is the probability that a crossing-over event will occur between two loci in meiosis. It is designated by the symbol θ . If two loci are not linked, i.e. are on different chromosomes or are very far apart on the same chromosome, then the chance that they segregate independently is 50% and therefore $\theta = 0.5$. If, however, in 19 out of 20 meioses the alleles at two loci segregate together, then they are on the same chromosome and they are said to be linked with $\theta = 0.05$.

The unit of measurement of linkage map distance between two loci is the morgan. One Morgan, or 100 centiMorgans (cM), is defined as the map distance in which an average of one crossover per chromosome strand occurs. Over very small distances, the probability of more than one crossover is negligible. Thus, for example, if two loci are one cM apart, then a crossover would be expected to occur between them once in every 100 meioses, and $\theta = 0.01$. The human genome is estimated by recombination studies to be 3,000cM in length. The physical length of the human genome is approximately 3×10^9 base pairs (bp), so on average, one cM corresponds to 10^6 bp. The relationship between genetic distance and physical length is not, however, linear because crossing-over is a nonrandom process with some regions being recombination "hotspots" and vice versa; also, recombination occurs more frequently in female than in male meioses.

2.4 Lod Score

In order to test the hypothesis that two loci are linked, a series of likelihood ratios are calculated for different values of the recombination fraction θ , ranging from $\theta = 0$ (i.e., tightly linked) to $\theta = 0.5$ (i.e., unlinked). The likelihood ratio at a given value of θ equals the chance of the observed data occurring if the loci are linked at the recombination value of θ divided by the chance of the observed data occurring if they are unlinked ($\theta = 0.5$). The logarithm to the base 10 of this ratio is known as the Lod or Z score, i.e., $\text{Lod}(\theta) = \log_{10} [L\theta/L(0.5)]$. Logarithms are used because they allow the results of linkage studies from different families to be added together. A Lod score of 3, which means that there is a greater than 10^3 to 1 chance that the observed data are due to the loci being linked rather than unlinked, is taken as the level of significance confirming linkage (Terwilliger and Ott 1994).

2.5 Multipoint Linkage Analysis

Two-point linkage analysis is used to map a disease locus to a specific chromosome region. More precise mapping can be carried out by multipoint linkage analysis of a series of ordered polymorphic markers known to map to a particular region. This allows the most likely position of the disease locus relative to known markers to be estimated based on the location score, which is a multipoint Lod score (Fig. 3.2) (Terwilliger and Ott 1994). The next step is physical mapping to isolate/identify the gene responsible (Liang et al. 1998). The limit of resolution for defining a candidate region by linkage analysis is usually about 1 cM. Analysis of recombination events in individual families may allow further refinement of the interval containing the gene.

3. Difficulties in Locating Hearing Impairment Genes

The process of locating the position of a gene by linkage analysis has been, until recently, a daunting prospect. The first requirement is to collect families in which the disorder is segregating. While this is easy with some forms of syndromic inherited hearing impairment with distinctive physical features, it is not so simple with nonsyndromic sensorineural inherited hearing impairment. The well recognized genetic heterogeneity of inherited nonsyndromic sensorineural hearing impairment, with multiple genes being responsible for both autosomal dominant and autosomal recessive forms, means that special care is needed in collecting families for linkage studies. If linkage data from unrelated small nuclear families are used, efforts to localize a gene are likely to be unsuccessful because a different gene may be responsible for the hearing impairment in each family.

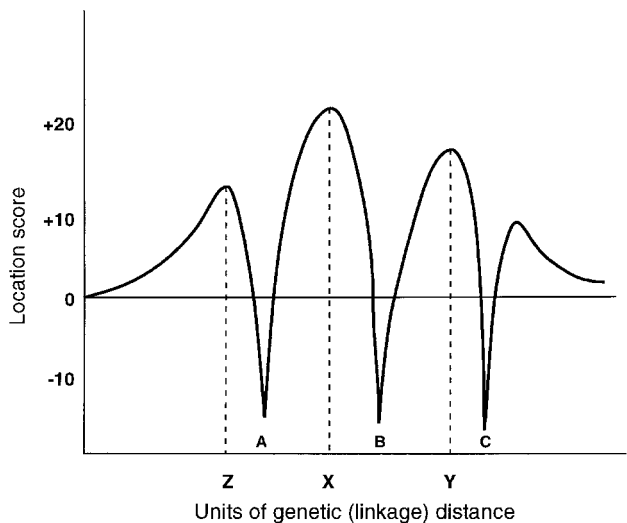


FIGURE 3.2. Multipoint linkage analysis. A, B and C represent the known linkage relationships of three polymorphic marker loci. X, Y and Z represent, in descending order of likelihood, the probable position of the disease locus. (Reprinted from Emery's Elements of Medical Genetics, 10th ed, Meuller, RF, Fig. 3.2, Copyright 1998, by permission of Churchill Livingston.)

In the case of autosomal dominant nonsyndromic sensorineural hearing impairment, single large families have been collected in which there are affected individuals in multiple generations in order to overcome this problem. An example of this approach was the analysis of the large Puerto Rican family with autosomal dominant low-frequency hearing impairment (Leon et al. 1981), which led to the identification of the first locus for autosomal dominant nonsyndromic sensorineural hearing impairment, *DFNA1*, on the long arm of chromosome 5 (Leon et al. 1992).

In the case of autosomal recessive nonsyndromic sensorineural hearing impairment, the problem is potentially even more difficult. In the majority of families with autosomal recessive nonsyndromic sensorineural hearing impairment, there are usually at most two or three affected siblings. Pooling the Lod scores from these families is very unlikely to demonstrate linkage. This problem has been overcome by use of the method of autozygosity mapping, which involves using samples from families in which the parents of affected offspring are consanguineous (Lander and Botstein 1987). Affected individuals in such pedigrees will have regions of their genome that are homozygous for polymorphic DNA markers because they were inherited from a common ancestor (Fig. 3.3). Identification of large consanguineous families from ethnic isolates means a single family can be sufficient to establish linkage (Mueller and Bishop 1993; Kruglyak et al.

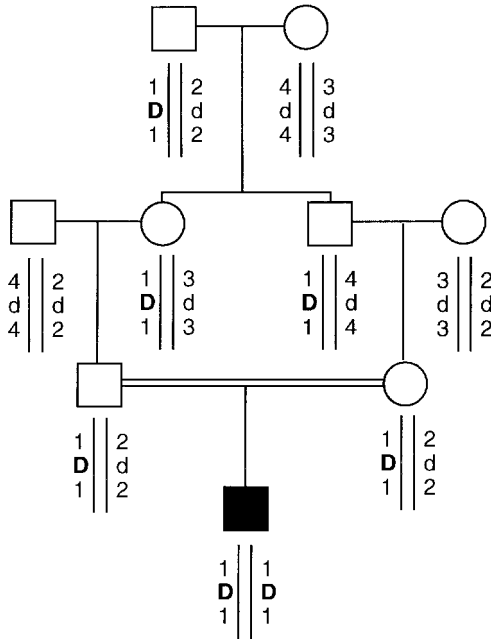


FIGURE 3.3. Diagrammatic representation of homozygosity for the disease allele (D) and flanking markers to a disease locus in the offspring of a first cousin union in autozygosity mapping.

1995). This approach has been utilized to identify the majority of the loci for autosomal recessive nonsyndromic sensorineural hearing impairment, e.g., mapping of the first locus for autosomal recessive nonsyndromic sensorineural hearing impairment, *DFNB1* (Guilford et al. 1994; Brown et al. 1996).

4. Genome Searches

In most instances, there are no clues to the chromosomal location of a gene, and genetic mapping involves the “shotgun” approach of a total genome search. This involves analyzing DNA samples from family members for a standard battery of approximately 300 polymorphic DNA markers evenly spread throughout the human genome. Until recently, this approach was fairly time-consuming and laborious. The advent of semi-automated computer-driven gel analysis of fluorescently labelled microsatellite markers means that, if the necessary number of samples from the families have been collected and the reaction conditions for the marker set are optimized, a genome search can be carried out in a matter of weeks, rather than years.

5. Candidate Regions

Occasionally, an individual with a single gene disorder is also found to have a structural chromosomal abnormality or rearrangement such as a deletion, inversion or translocation (Giersch and Morton, Chapter 3). In such individuals, the structural chromosomal abnormality or rearrangement is thought to have caused either the physical loss (deletion) of the gene or disrupted the gene, both of which could lead to a loss of function. The cytogenetic location of the structural abnormality or rearrangement constitutes a candidate region for the location of that gene, which can be confirmed by linkage studies. The report of an individual with typical features of the autosomal dominant disorder Waardenburg syndrome type I (WS1), in which sensorineural hearing impairment can occur, with a *de novo* chromosomal inversion on chromosome 2 at 2q35–q37.3 (Ishikiriyama et al. 1989), suggested that the gene for WS1 was located in that region. Subsequent linkage analysis confirmed this suggestion (Foy et al. 1990).

The mouse is the mammal that offers the best understanding of the genetics and biology of human inherited disorders and diseases. Comparison of the genetic maps of the mouse and humans have established conserved-linkage groups (Copeland et al. 1993; Dietrich et al. 1995). This has allowed a comparative map of mouse and man to be established, enabling identification of potential mouse models for the study of inherited human disease (Brown 1994). Over 100 mutations that affect the development or function of the mouse inner ear have been identified. In many cases, mouse-human homology has facilitated identification of genes for hearing impairment (Steel, Chapter 8).

6. Physical Mapping

Once linkage analysis assigns a gene to an appropriately small interval of a specific region of a chromosome, the next stage is to carry out physical mapping of the region of interest to refine the localization of the gene. This is done by identifying overlapping clones from a library with large human genomic DNA inserts to form what is known as a contig. In the first instance, this is often done with yeast artificial chromosomes (YACs) and once the interval of interest is narrowed down, with bacterial artificial chromosomes (BACs) and/or bacteriophage P1 artificial chromosomes (PACs). Up to two Mb of DNA can be cloned into YACS, whereas BACs typically contain DNA inserts up to 150 kilobases in size.

6.1 Contigs

Originally the first stage of physical mapping was to screen YAC libraries (Albertson et al. 1990; Anand et al. 1990) to identify by PCR those clones

that contain polymorphic markers known to map to the interval in the region of interest. Now, because of the progress of the Human Genome Project, YAC contigs of most regions of the genome are available online. Identification of unique DNA sequences in the YAC clones allows their order to be determined leading to a physically contiguous series of overlapping YAC clones (contig).

A concern with YACs is that the DNA inserts are often chimeric, i.e., contain DNA fragments from noncontiguous regions of the genome. In addition, YAC clones are often unstable, undergoing rearrangements in propagation. For this reason, once the region containing a gene of interest has been narrowed down sufficiently, bacterial artificial chromosomes (BACs) and/or bacteriophage P1 artificial chromosomes (PACs), which replicate stably, are used to establish a contig of the interval of interest.

Identification of a gene in a contig may occur quite rapidly if the region of interest has previously been screened in a different study. For example, the *DFNB3* locus mapped to chromosome 17p11.2, a region that was known to contain the gene for hereditary motor sensory neuropathy type 1a (*CMT1A*) (Murakami and Lupski 1996) and was also the location of the microdeletions responsible for Smith–Magenis syndrome (Juyal et al. 1996). This allowed screening of genes that had previously been found in that region (Murakami et al. 1997), one of which was the homologue of the gene responsible for the mouse deafness mutant *shaker-2* (Liang et al. 1998).

6.2 Transcript Mapping

Once a physical contig is established for a particular region known to contain a gene of interest, the next step in the mapping process is the identification of expressed gene sequences or transcripts within that region (Giersch and Morton, Chapter 3).

6.2.1 Expressed Sequence Tags (ESTs)

Partial sequences of genes identified from cDNAs are known as expressed sequence tags (ESTs) (Adams et al. 1991). More than 50,000 human ESTs have been identified, many of which have been mapped and deposited in the DNA sequence database, dbEST (Boguski 1993; Boguski and Schuler 1995; Schuler et al. 1996). Recently 30,000 of these ESTs have been assembled in an integrated map (Deloukas et al. 1998), nearly twice as many as three years earlier (Berry et al. 1995). This resource contains most of the genes that encode proteins of known function. By selecting the chromosomal region of interest in the database, ESTs mapping to that region can be identified.

ESTs, however, represent only partial sequences of genes and it is necessary to identify the full sequence to be able to reliably screen for mutations in a specific gene. This is done by screening a cDNA library with the

ESTs to identify close to full-length cDNA clones of the transcript. These will usually be in the region of one to four kilobases in length, as opposed to most ESTs, which are generally 300 to 400 bp in length. The technique of rapid amplification of cDNA ends (RACE) may then be applied to generate the missing portions of the cDNA. This technique takes advantage of the polymerase chain reaction (PCR) to amplify the missing sequence using mRNA from a specific tissue in which the gene of interest is expressed (Frohman et al. 1988).

6.2.2 Exon Amplification/Trapping

Not all genes are represented in sequence databases. Thus, alternate approaches may be necessary to identify candidates in the region of interest. Exon amplification/trapping relies on the fact that the coding regions of most eukaryotic genes, exons, are separated by noncoding intervening sequences, introns. The production of mature mRNA involves the removal of the introns by the process of mRNA splicing to produce continuous coding sequence for protein translation. The exon/intron boundaries contain 5' donor and 3' acceptor splice site consensus sequences. Exon trapping utilizes these sequences to identify clones containing exons. Expression cloning vectors containing the DNA sequence are used to transfect a suitably modified eukaryotic cell line, resulting in transcription of the inserted target DNA into RNA, which then undergoes splicing. The production of an abnormal-sized splice product after insertion of a target DNA fragment indicates the presence of an exon in the cloned DNA fragment (Duyk et al. 1990).

A major advantage of exon trapping is that it is not dependent on the expression of the gene. Exons from genes expressed in specific tissues, or at particular stages of development, are isolated with the same efficiency as genes that are more widely expressed (Church et al. 1994). Disadvantages of exon trapping are that genes with single exons and exons in the 3' and 5' portions of the gene will be missed. Specialized vectors have been devised to help overcome this problem (Krizman et al. 1995).

6.2.3 Phenotype Rescue

More recently, "phenotype rescue" in the mouse has helped to rapidly narrow the interval within which a gene of interest is located. In this procedure, BAC clones from the contig to which the gene has been mapped are injected into fertilized mouse oocytes that are homozygous for a mutation in this gene (e.g., a gene causing hearing impairment). Identification of a transgenic mouse with normal hearing (i.e., an offspring for which the phenotype reverts to normal or is "rescued"), suggests that the injected BAC clone contains the gene. This approach was used to identify *Myo15* as the defective gene in the *shaker-2* mouse (Probst et al. 1998). Almost immediately, mutations in the human homologue, *MYO15*, were shown to be

associated with one form of nonsyndromic sensorineural hearing impairment/deafness, *DFNB3* (Wang et al. 1998a).

7. Positional Candidate Gene Cloning

If a gene for a disorder is mapped to a specific chromosomal region, a known gene mapping to that interval is a positional candidate by virtue of its location. One of the first examples of the use of this approach was in Waardenburg syndrome type 1 (WS1). It was recognized that the region of human homology for the mouse mutant known as *Splotch* (*Sp*) (which is deaf and has areas of depigmentation similar to that seen in persons with Waardenburg syndrome) was 2q35–q37.3. A structural rearrangement of this region of chromosome 2 had previously been reported in an individual with WS1 and subsequent linkage analysis confirmed this location (Foy et al. 1990). The *Pax3* gene in the mouse and the human homologue *PAX3* mapped to the same intervals as the *Sp* and WS1 loci suggesting positional candidate genes for both disorders. This was confirmed by the identification of mutations in the *Pax3* gene in the *Sp* mutant and in the *PAX3* gene in WS1 patients (Tassabehji et al. 1992; Baldwin et al. 1992).

8. Mutation Detection

One of the essential prerequisites for confirmation that a gene causes an inherited disorder or disease is the identification of DNA sequence differences that are present in affected individuals, but not in unaffected individuals from the general population. In other words, differences that are normal sequence variation between individuals need to be distinguished from deleterious mutations that result in inherited disorders.

8.1 Prioritizing Candidate Genes for Mutation Detection/Screening

Determining which candidate genes to screen for mutations may not be obvious. A first step is to examine those that have been characterized. The function of the proteins encoded by these genes may suggest that some are more likely candidates than others. For example, *MYO15* was considered a probable candidate gene for *DFNB3* because mutations in another unconventional myosin, *MYO7A* had been associated with inherited hearing impairment (Wang et al. 1998a). If function is unknown, evidence of expression in the inner ear helps in prioritizing candidates for mutation screening (Giersch and Morton, Chapter 3). For example, a transcript (*COCH*) from a fetal cochlear cDNA library (Robertson et al. 1994) was localized to the region of the long arm of chromosome 14 to which the *DFNA9* locus had

been mapped (Robertson et al. 1997). Subsequent sequence analysis identified mutations in *COCH* in hearing impaired *DFNA9* family members (Robertson et al. 1998).

8.2 Mutation Detection/Screening Methods

Once a potential candidate gene for a disorder has been identified, a variety of different complementary methods can be used to screen for mutations. Each method has its own advantages and disadvantages.

8.2.1 Single-Stranded Conformational Polymorphism (SSCP)

Following denaturation, PCR products fold into a three-dimensional structure in non-denaturing conditions through the influence of sequence-specific intramolecular bonds. If there is a sequence difference between DNA strands, the resulting three-dimensional structure (conformation) may have a different mobility through a nondenaturing polyacrylamide electrophoretic gel than if the DNA strands are identical (Orita et al. 1989). Sequence variation detected in this way is known as single-stranded conformational polymorphism (SSCP) (Fig. 3.4). The optimal DNA fragment size for mutation detection by this technique is 200 to 300 bp in length. The sensitivity of SSCP for detecting mutations varies with factors such as temperature, the strength of cross-linking, and ionic strength of the gel. In addition, SSCP is not efficient at detecting mutations in DNA fragments that are larger than 350 bp.

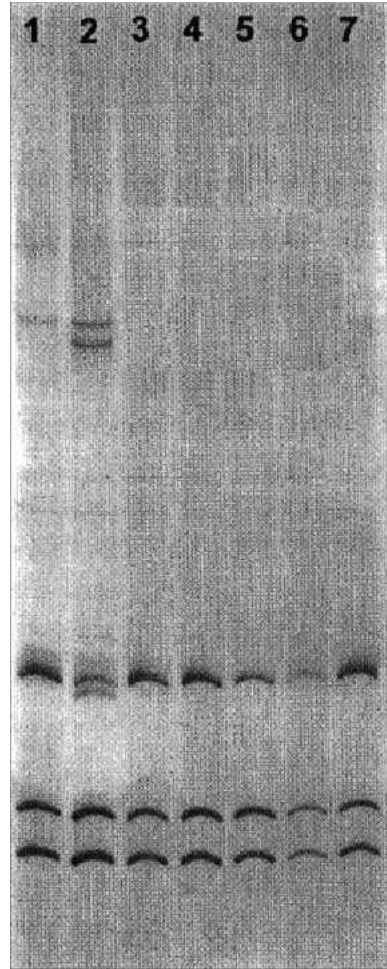
8.2.2 Denaturing Gradient Gel Electrophoresis (DGGE)

Denaturing gradient gel electrophoresis (DGGE) relies upon differences in mobility between normal and mutant DNA on an electrophoretic gel with a gradient of increasing denaturant concentration. Migration of double-stranded DNA fragments through the gel will continue until the strands of the DNA duplexes begin to separate because of the presence of the denaturant (Fischer and Lerman 1979). Addition of a 30 to 40 nucleotide GC-rich sequence (a GC clamp) to the 5' end of the primers used to generate the DNA fragments for analysis makes DGGE a highly sensitive mutation-detection method (Myers et al. 1985; Sheffield et al. 1989), but also results in its becoming relatively expensive because of the need to synthesize longer PCR primers.

8.2.3 Heteroduplex Analysis

In heteroduplex analysis, PCR products generated from both mutant and normal DNA templates are mixed, heat denatured, and allowed to cool to room temperature. This results in the formation of heteroduplexes of the wild type and mutant DNA sequences. These heteroduplexes often differ

FIGURE 3.4. SSCP analysis of PCR products of exon 2 of the coding region of the *Cx26* gene of DNA samples from persons with nonsyndromic hearing impairment showing a mobility shift/doublet in an individual with the 310de114 mutation in the *Cx26* gene in the sample in Lane 2.



in mobility from homoduplexes on polyacrylamide gel analysis (Nagamine et al. 1989). This is thought to be due to sequence-dependent conformational changes in the double-stranded DNA duplex. Heteroduplex analysis has a similar sensitivity to SSCP for DNA fragments less than 300 bp in size (White et al. 1992).

8.2.4 Cleavage Mismatch

Cleavage mismatch involves adding the chemicals hydroxylamine and osmium tetroxide, which react with free cytosine and thymine nucleotides, respectively. The DNA template is denatured, then hybridized with a single-stranded radiolabeled DNA probe. Any mismatched cytosine or thymine nucleotides will be exposed and susceptible of reaction with the hydroxy-

lamine and osmium tetroxide. Such sites are detected by the addition of piperidine, which results in cleavage of the DNA at these sites. The technique identifies mismatches on either strand, and thereby indirectly detects adenine and guanine mismatches through their complementary nucleotides on the other strand (Cotton et al. 1988). Chemical cleavage works for longer DNA fragments than SSCP, DGGE, and heteroduplex analysis and it also has the advantage that it provides information about the location of the mutation. Adaptation to fluorescent-based detection systems (Ellis et al. 1998), as well as use of less toxic chemicals (Roberts et al. 1997) and enzymatic methods of chemical cleavage (Rzhetsky et al. 1996; Smith and Modrich 1996; Kortenkamp et al. 1997), may increase the use of chemical cleavage as a mutation-detection method.

8.2.5 Sequence Analysis

DNA sequencing is the gold standard of mutation detection, but it is expensive and laborious, especially if the gene being sequenced is large. However, advances in automated DNA sequencing technology are making sequence analysis the method of choice for mutation detection. In addition, ongoing developments in DNA chip technology will permit rapid, automated, large-scale mutation detection once a gene has been identified.

9. Approaches to Confirming that a Detected Mutation Causes Hearing Impairment

While identification of mutations in affected individuals in a candidate gene provides strong evidence that it is responsible for inherited hearing impairment, further evidence can be provided in a number of different ways.

9.1 Prediction of the Likely Functional Consequences of Mutations

Analysis of the functional consequences of specific mutations can provide compelling evidence that a particular gene is responsible for inherited hearing impairment. For example, deletion of the whole gene, as has been reported in some patients with the branchio-oto-renal syndrome (Abdelhak et al. 1997; Kumar et al. 1998), would be expected to lead to loss of function. Alternatively, a nonsense mutation, or a frameshift mutation that generates a stop codon, especially if it occurs in the 5' portion of a gene, leads to a severely truncated protein, which is likely to have severe functional consequences. A number of mutations in the connexin 26 (Cx26) gene are of this type (Avraham and Hasson, Chapter 2).

The likely functional consequence of missense mutations cannot be predicted reliably in some instances, and may require knowledge of the

structure and function of the protein. Mutations which result in nonconservative amino acid substitutions in a part of the protein that is critical to its function, or in regions known to be conserved between species, are likely to have significant effects. Examples include a T to G missense mutation in a family with Pendred's syndrome, which leads to substitution of Cys for Phe at a highly conserved position in the protein pendrin (*PDS*) (Everett et al. 1997), and the single nucleotide missense mutations reported in the *POU* homeodomain of the *POU3F4* gene in individuals with the X-linked form of mixed deafness *DFN3* (Kok et al. 1995).

9.2 Analysis of Control Samples

Supportive evidence that a DNA sequence variant is likely to be a mutation, rather than a normal polymorphism, is provided by the absence of the sequence variant in an appropriate number of normal control individuals. The M34T sequence variant in the *Cx26* gene reported in association with autosomal dominant sensorineural hearing impairment (Kelsell et al. 1997) occurs in a small but significant proportion of the normal population; this suggests that it might not be of functional significance, but instead a normal polymorphic variant (Scott et al. 1998; Kelley et al. 1998). However, in vitro functional studies (Section 9.3) provide support for the association of this mutation (101T to C) with hearing impairment.

9.3 In Vitro Functional Studies

In vitro functional assays of a protein can reveal the effect of known or introduced mutations in a gene. For example, mRNA generated from wild type and variant *Cx26* gene sequences was expressed in *Xenopus* oocytes to measure gap junction channel activity. Analysis of the 101T to C sequence variant in the *Cx26* gene (Kelsell et al. 1997) indicated that it was likely to be a mutation, rather than a polymorphic variant (White et al. 1998). However, care needs to be taken in the interpretation of the results of these types of studies, since it is possible that they might not reflect function in vivo.

9.4 Immunohistochemical/In Situ Hybridization Studies

Further evidence that a mutation in a gene may be responsible for inherited hearing impairment can be obtained by examining the expression of the gene at the mRNA or protein level in appropriate tissues using immunohistochemical or in situ hybridization studies. For example, *Cx26* expression is found in the stria vascularis, basement membrane, limbus and spiral prominence of the cochlea using murine monoclonal antibodies to *Cx26* (Fig. 3.5) (Kelsell et al. 1997).



FIGURE 3.5. *Cx26* immunofluorescence staining of the cochlear duct. Rabbit polyclonal anti-*Cx26* staining showing expression in the stria vascularis, basement membrane, limbus and spiral prominence (magnification, 16 \times). (Reprinted with permission from Nature (387:80–83). Copyright 1997 Macmillan Magazines Limited.)

9.5 Transgenic/Knockout Models

The ability to introduce targeted mutations in a gene by homologous recombination in the mouse allows the possibility of confirming the phenotypic consequences of mutations in a gene for inherited hearing impairment or deafness (Friedman and Ryan 1992; Friedman 1996; Faerman and Shani 1997). Examples are the analysis of the role of the *POU*-domain factor *Brn-3c* gene in auditory and vestibular hair cell development (Xiang et al. 1997) and the knockout mouse model for Alport syndrome (Cosgrove et al. 1988).

9.6 New Approaches Resulting from the Human Genome Project

The availability of human DNA sequence information has allowed the development of a new cloning strategy for identifying expressed sequences. If a gene is mapped to a relatively small interval, genomic clones from the contig covering that region can be subcloned and sequenced. The resulting sequence information can be compared with that in a number of sequence databases, such as the GenBank BLAST Search database (<http://www.ncbi.nlm.nih.gov/BLAST/>) to identify sequences with homol-

ogy to known genes and proteins (Altschul et al. 1990; Altschul et al. 1994). In addition, computer programs such as Gene Recognition Analysis and Internet Link (GRAIL) (<http://www.ncbi.nlm.nih.gov/genemap>) and GENSCAN can be used to interrogate sequence data for the presence of features that suggest genes, such as splice-site consensus sequences.

9.6.1 In Silico Cloning

The burgeoning field of bioinformatics is a rapidly growing discipline as a consequence of the large amount of DNA sequence information that is available. It is now possible to clone genes from sequence data analysis alone; this has been called “in silico cloning.” Two genes for inherited hearing impairment, the gene for Pendred’s syndrome (Everett et al. 1997) and the *DFNA5* gene (Van Laer et al. 1998), have been identified this way. This approach will almost certainly result in the identification of an increasingly greater proportion of the genes for inherited hearing impairment with the availability of the EST and human genome DNA sequence data.

10. Summary

Over the past six years, linkage studies of families with autosomal dominant, autosomal recessive and X-linked syndromic and nonsyndromic sensorineural hearing impairment have resulted in mapping of 30 autosomal dominant, 28 autosomal recessive and 5 X-linked loci for nonsyndromic hearing impairment. Physical mapping and the use of a variety of cloning approaches over the same time period have led to the identification of 18 of these genes. Further progress in the mapping and cloning of genes for inherited hearing impairment can only accelerate in the future.

There is an urgent need to document in detail the phenotypic features in persons with inherited hearing impairment, and to correlate them with the genotypic findings, both the particular gene involved and the specific mutation responsible. In addition to the audiological findings (severity of hearing impairment, age of onset, progression, audiological configuration, etc.), the description of the phenotype should include documentation of the presence or absence of associated vestibular abnormalities, and/or neuroradiological findings in the inner ear. By this means, the results of the research findings will rapidly translate into the development of directed and appropriate mutation testing, not only for individuals with or at risk for inherited hearing impairment, but also for the most common scenario, namely the sporadic child with nonsyndromic sensorineural hearing impairment, for whom limited diagnostic tests are available.

The identification of the normal function of the products of the genes responsible for inherited hearing impairment will help us understand how mutations in them result in hearing impairment, as well as identifying

environmental factors that cause hearing impairment, with the prospect, in time, of therapeutic intervention.

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4

Genetic Epidemiology of Deafness

WALTER E. NANCE and ARTI PANDYA

1. Introduction

Genetic deafness is a classic example of multilocal genetic heterogeneity in which a common phenotype can be caused by major genes at a large number of loci. This insight into the true nature of the phenotype is of relatively recent origin, and the major current emphasis of research has been on the identification of the specific genes and mutations that contribute to hearing loss. The molecular approach to the genetic epidemiology of deafness has been a spectacular success. Nevertheless, a comprehensive understanding of the forces that have shaped the temporal and geographic distribution of deafness phenotypes will require epidemiological data on secular trends in the incidence of specific phenotypes, as well as knowledge about the mating structure of the population in question. The goal of this chapter is to provide a foundation for such an understanding. The model of multilocus heterogeneity, together with highly specific genetic and environmental interactions, is emerging as an explanation for the hereditary component of deafness. Similar models are likely to be applicable to other complex diseases.

2. Historical Perspective of the Epidemiology of Deafness

Deafness has been a familiar part of human experience since the dawn of recorded history. For example, special provisions and protections were provided for the deaf in both Roman and Hebrew law (Bender 1970). In China, Han Yu, the famous historian of the T'ang Dynasty, may have anticipated the recognition of atypical Usher syndrome by more than a millennium when he lamented the gradual loss of his sight and hearing before his 50th birthday. It was not until the 16th century, however, that a Benedictine monk named Pedro Ponce de Le'on clearly demonstrated the educability of the deaf. By developing a method of teaching the deaf scions of the Spanish

aristocracy to read and even speak, he clearly demonstrated the fallacy of the Aristotelian concept that the mutism that typically accompanied deafness was the result of a primary defect of the tongue.

2.1 *Pre-Mendelian Era*

Interest in the epidemiology of deafness began in the 19th century with formal censuses of the deaf being conducted in many European countries, as well as in the United States. Although Mendel's seminal work on genetics was not yet known by the scientific community, the familial aggregation of deafness was clearly recognized, and there was considerable speculation about the significance of consanguinity as a cause of congenital abnormalities including deafness. For example, Mygind (1894) surveyed the deaf in Denmark and reported the reduced reproductive fitness of the deaf, as measured by the relative frequency of marriage in deaf males (41%) and females (24%) compared with the hearing population. He also documented the high frequency of assortative mating (29%) among the deaf.

Towards the end of the century, as more schools for the deaf were established and the social and economic prospects of the deaf improved, the proportion of the deaf population who married and reproduced began to increase. These trends prompted debate about the potential effects of marriages among the deaf on the incidence of deafness in future generations. Thus, Mygind (1894) reported no cases of deaf-mutism among 183 children of marriages in which one or both parents were deaf. Similarly, in 1881 the commissioners of the Irish census reported that "it appears evident that the question of deafness and dumbness in the parents has no influence on the propagation of the defect" (Fay 1898).

In this country, however, Alexander Graham Bell expressed concern that "if the laws of heredity that are known to hold in the case of animals also apply to man, the intermarriage of congenital deaf mutes through a number of successive generations should result in the formation of a deaf variety of the human race" (Bell 1883). Bell collected data on more than 5,000 deaf alumni of six schools for the deaf in the United States and showed that 29% had deaf-mute relatives. Commenting on the data he collected in an article in the *Memoirs of the National Academy of Science* (1883), Bell wrote:

"... but can it be accidental that there should have been admitted into one institution eleven deaf-mutes of the name 'Lovejoy' seven of the name 'Derby' and six of the name 'Mayhew'. What interpretation shall we place upon the fact that groups of deaf mutes are to be found having such names as 'Blizzard', 'Fahy', 'Hulett', 'Closson', 'Brasher', 'Coper', 'Gortschlag', etc.? Such names are by no means common in the community at large, and the influence is irresistible that in many cases the recurrences indicate blood relationships among the pupils."

Prompted by Bell's concerns, E.A. Fay, a professor at Gallaudet College, initiated the systematic collection of pedigree data on the occurrence of

deafness in the parents, children and siblings of 4,471 marriages occurring between 1803 and 1894 in which one or both of the partners were deaf (Fay 1898). Overall, Fay found that only 9.7% of these marriages produced deaf offspring. Although this frequency was far greater than the incidence among hearing couples, he also emphasized that these marriages were “far more likely to result in hearing offspring than deaf offspring.” Fay also showed that marriages involving parents with “congenital” as opposed to “adventitious” deafness appeared to have a higher incidence of deaf offspring and, in his discussion of the effects of consanguinity, Fay provided further evidence that he recognized the heterogeneous nature of deafness. In his monumental study *Marriages Among the Deaf in America*, Fay (1898) wrote:

“Now if a person who is deaf from one anomaly or disease of the auditory organs, or of the nervous system marries a partner who is deaf from some different and unconnected pathological condition, the law of heredity under consideration should not lead us to expect any intensification of the liability to transmit the defect, for the characteristics existing in the two partners are not the same. Although both partners are deaf, these marriages are not a union of ‘like with like’ from a physiological point of view, and the law does not apply to their case. On the other hand, where the physical condition that results in deafness is the same in both partners as for instance it probably is in consanguineous marriages of deaf persons—we should expect the liability of deaf offspring to be intensified by their union.”

Fay was able to identify a small sample of 31 consanguineous marriages among the deaf. He found that 45% of the marriages had at least one deaf child, and that 30% of the offspring from these marriages were deaf. Fay concluded that consanguineous marriages were very likely to have deaf offspring whether the deafness was congenital or adventitious, whether there were other deaf relatives, and whether only one or both parents were deaf. Without the benefit of Mendel’s yet to be rediscovered theory of the gene, Fay’s intuition about the significance of consanguinity is remarkable and anticipates the extensive use of such marriages to map genes for deafness by nearly a century.

2.2 *Post-Mendelian Era*

Following the recognition of Mendel’s work, the Fay data set has been repeatedly reanalyzed using progressively more complex genetic models. In 1910, Hammerschlag, who was familiar with the transmission of recessive deafness in the mouse, attempted to explain human deafness by a recessive gene at a single locus. However, even when the data set was limited to deaf by deaf (DxD) matings in which each parent had three or more deaf siblings, he found that more than half the offspring were hearing, instead of the expected 100% deaf. Later, Kraatz (1925) proposed a two-locus model in which deaf individuals were assumed to be homozygous at one or both

of two loci. This model could readily explain how deaf by deaf matings could either produce all normal (complementary matings) or all affected offspring (noncomplementary matings), but could not readily explain the substantial frequency of deaf by deaf matings that produced both hearing and deaf offspring. As an alternative, Dahlberg (1931) proposed an even more complex genetic model in which affected individuals were assumed to be carriers, simultaneously, of dominant genes at three loci, as well as homozygotes at another locus (to explain the increased consanguinity). By the appropriate selection of gene frequencies, he showed how the observed proportions of affected individuals in various mating types could be explained. Under Dahlberg's ingenious model, the high frequency of sibships with only one deaf child is explained by mating types such as (A/a, B/b, C/c, d/D) \times (a/a, b/b, c/c, d/D), which have a 1/32 segregation ratio, rather than by sporadic environmental causes. This model, however, does not provide a mechanism for dramatic increases in the frequency of sibships with only one deaf child, as occurs, for example, with a rubella epidemic.

In 1956, Stevenson and Chessman collected family data on 700 probands or affected-index cases from Ireland who became deaf before six years of age, a sample that included virtually no recognized cases of rubella deafness. For the offspring of consanguineous matings, a recessive model that assumed complete penetrance for the deafness given homozygosity for the deleterious gene, provided an excellent explanation for the data. In these families, there was no evidence for prenatal loss of deaf fetuses, or for failure of the genotype to be expressed (reduced penetrance) because of modifier genes or other factors. However, among the offspring of non-consanguineous hearing by hearing (H \times H) matings, there was a 25% excess of cases that appeared to be sporadic or nongenetic. Also, 21 of 32 D \times D matings that were ascertained through the parents had hearing offspring, and only five had deaf offspring exclusively. Because these families were identified (ascertained) through deaf marriage partners regardless of their children's hearing status, they included families with hearing offspring exclusively. (In contrast, ascertainment through offspring would only have included families with at least one deaf child). The authors recognized their findings as an indication of the existence of two or more recessive loci.

If all deafness was caused by recessive mutations at a single locus, all the children of deaf by deaf matings would be expected to be deaf. If deafness was caused by two equally frequent recessive genes, and deaf by deaf marriages occurred at random, only one half of the marriages would be expected to produce deaf offspring exclusively. The remaining "complementary" matings between individuals with different recessive genes would produce only hearing offspring. This hypothesis was consistent with the frequency of consanguinity, which was much higher in Stevenson and Chessman's (1956) sample than would have been expected if all the cases of recessive deafness had been caused by mutations at a single locus.

Among the 309 HxH matings, 11.7% were consanguineous. If only “genetic” cases were included (that is, those with a nongenetic etiology, such as rubella, were excluded from the analysis), the adjusted rate for recessive pedigrees would have been 13.2%. Chung and Morton (1959) subsequently analyzed Stevenson and Chessman’s data using maximum-likelihood methods and estimated that the proportions of sporadic, recessive and dominant cases were 0.22, 0.56 and 0.22, respectively. Using the theory of detrimental equivalents, they also estimated that genes at 36 ± 12 independent recessive loci contribute to the phenotype. Rose (1975; 1977) applied Morton’s methods to the 1,722 proband matings and 2,355 proband sibships in the Fay data set, together with 12,661 nuclear families collected as part of an Annual Survey of Hearing Impaired Children in 1968, and family histories on 482 students at Gallaudet University. The overall estimates of the proportions of genetic cases were 0.55 in the Fay data, 0.51 in the National Survey and 0.76 among the Gallaudet students. Recessive transmission accounted for 88%, 85% and 78% of the genetic cases respectively. Among the HxH sibships in the Fay data, the overall and adjusted rates of consanguinity were 7.1% and 15.4%. In the two large nationwide data sets, which were collected 100 years apart, the estimated proportion of genetic cases was remarkably consistent, even though the latter is known to have included a large cohort of patients with rubella deafness. The higher proportion of genetic cases, and cases showing apparent dominant transmission among the high-achieving Gallaudet student population, is of interest. Most of these students have at least one deaf parent, and at home as well as at Gallaudet ASL was probably the language of choice. Thus, as might be expected, a familiar language and culture facilitate academic excellence.

Fay’s data set included 1,299 in which both parents were deaf. Among these, the estimated proportion of non-complementary matings (that is, those that can produce only deaf offspring) was 0.042 ± 0.007 , while the estimated proportion of complementary matings (that is, all offspring are hearing) was 0.875 ± 0.17 . Non-complementary matings refer to those between individuals with the same type of genetic deafness, while complementary matings refer to those between individuals with different types of genetic deafness. Genetic analyses of other large data sets have been reported by Macklin et al. (1946), Sank (1969), Chung and Brown (1970), Furusho (1957), Mori (1959), Marazita et al. (1993) and Liu et al. (1994). The study by Liu et al. (1994) is particularly noteworthy because it involved a clinical survey of 126,876 individuals drawn by a stratified random sampling procedure from the 104 million citizens of Sichuan province in China in 1986 to 87. In the sample, 236 individuals were found to have a hearing loss of 90 dB or more. The overall prevalence of profound deafness was 0.82 per 1,000 and ranged from 0.7 per 1,000 in the predominant Han ethnic group 0.0 among 2,933 Tibetans, to 6.6 per 1,000 among 1,968 members of the Lisu minority group. The prevalence ranged from about 0.5 per 1,000 for subjects less than 30 years of age, to a high of 1.8 per 1,000 for those

between 30 and 45 years of age. A segregation analysis that included 104 of the cases showed that 71% were genetic, while the remainder were sporadic, being determined largely by nongenetic causes associated with a negligible chance of recurrence within families. Among the genetic cases, 4.2% were associated with distinctive clinical features that permitted the diagnosis of a syndrome, while 89% were nonsyndromic-recessive and 6.8% nonsyndromic-dominant. The reproductive fitness of these cases was estimated to be 0.4 relative to hearing siblings.

The existence of etiologic heterogeneity with both genetic and environmental causes has been a consistent feature of studies of deafness. Among genetic cases, recessive transmission predominates, but the observed and adjusted frequencies of consanguinity are much higher than would be expected from the incidence of the phenotype, yielding estimates of the number of recessive loci that have ranged from 36 to 103, or higher (Chung and Brown 1970; Morton 1991). Data on marriages among the deaf show strong evidence for assortative mating in many but not all countries (e.g., India). When DxD matings have been studied, segregation analysis shows that relatively few couples are capable of producing only affected offspring, a finding that is inconsistent with the assumption that most cases of deafness are caused by recessive mutations at a single locus. Rose (1975; 1977) showed that the observed proportion of non-complementary matings was consistent with the assumption that recessive deafness is caused by equally frequent mutations at about 10 loci. The fact that higher estimates are obtained from consanguinity analysis indicates that the recessive phenotypes are not equally frequent, a conclusion that has been amply verified by recent discovery of the high proportion of recessive deafness that can be attributed to mutations in the connexin 26 gene.

3. Genetic Epidemiology of Deafness

The incidence of profound deafness in the United States is about 0.8 per 1,000 births (Bodurtha and Nance 1988). If lesser degrees (>30 dB) of loss, or unilateral or conductive losses are included, the incidence at birth or early infancy may be as high as 1.5–6 per 1,000. These rates can be influenced greatly by temporal or geographic variation in the frequency of recognized environmental causes including pre- or postnatal infections, such as rubella, cytomegalic inclusion body virus (CMV), otitis media, meningitis, prematurity, trauma, kernicterus, and exposure to ototoxic drugs. Despite the introduction of rubella immunization programs, the congenital rubella syndrome remains an important cause of deafness. Studies of infants with congenital deafness have also suggested that as many as 12% may be attributable to prenatal CMV infections (Peckham et al. 1987). Aminoglycoside ototoxicity provides a good example of geographic variation in the causes of deafness. Because of the widespread use of these antibiotics in infancy and

childhood, ototoxicity is one of the commonest causes of deafness in Mongolia and China (Pandya et al. 1997; Hu et al. 1991).

A dramatic example of temporal variation in the incidence of deafness is provided by the last rubella pandemic. In the United States alone, more than 5,000 children were deafened by prenatal exposure to the virus. Examination of the birthdates of children at the Maryland School for the Deaf (Figure 4.1) reveals a remarkably high concentration during the fall and spring of 1964 to 65. During this period, there was also a marked increase in the proportion of sibships with only one deaf child and a corresponding decrease in the estimated proportion of genetic cases from about 0.5 to 0.1 (Bieber 1981). Rubella deafness is thus an entity in which the birth date can be an important clue to the etiology. Clearly, any attempt to partition deafness into its genetic and environmental causes may lead to inconsistent results if there is unrecognized secular or geographic variation in either the environmental or genetic causes of deafness.

3.1 Syndromic Deafness

During the past three decades, dramatic progress has been made in the clinical delineation of more than 150 forms of syndromic deafness (Konigsmark 1976). In a comprehensive clinical survey, Fraser (1976) estimated that a specific form of syndromic deafness could be recognized in 20 to 25% of the genetic cases. In many important deafness syndromes, such as those of

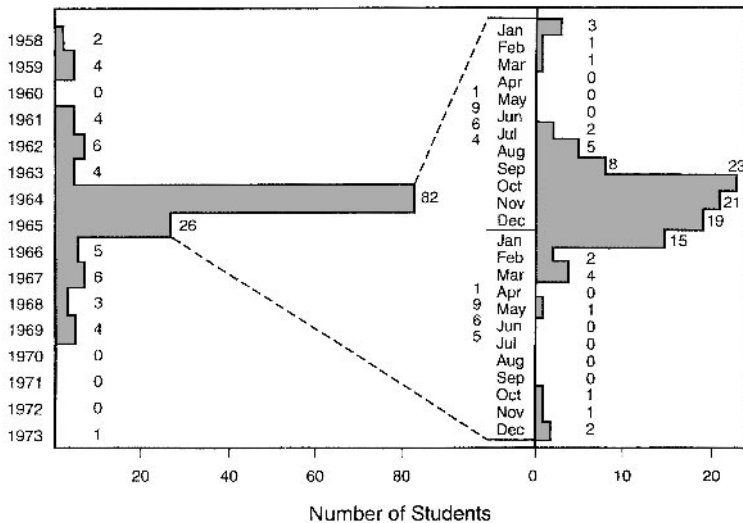


FIGURE 4.1. Birth dates of students at Maryland School for the Deaf by year and month showing cases resulting from phrasing rubella epidemic born in fall of 1964 and spring of 1965. (Data from Bieber 1981.)

Waardenburg (1951), Pendred (1896), Usher (1914), Treacher Collins (1900), Jervelle and Lange-Nielsen (1957) and Nance (1971), Mendelian transmission is well established and has allowed the mapping, demonstration of heterogeneity and/or cloning of more than 20 relevant genes. Distinctive audiologic or otolaryngologic characteristics, such as low-frequency (Vanderbilt University Hereditary Deafness Study Group 1968), high-frequency (Nance and McConnell 1974), or progressive hearing loss (Cremers 1979), or the presence of distinctive vestibular, cochlear or ossicular abnormalities, can also be used to characterize different forms of genetic deafness.

3.2 *Non-Syndromic Deafness*

Dramatic advances have also been made during the past decade in mapping and cloning human genes for nonsyndromic deafness (Mueller, VanCamp, and Lench, Chapter 4). Many factors have contributed to this progress. These include the availability of increasingly dense maps of highly polymorphic markers, and knowledge of mouse homologs for comparative gene mapping. In addition, sophisticated methodologies and linkage programs have been developed which exploit data on sib pairs (Penrose 1935; Kruglyak and Lander 1995), consanguineous probands (Lander and Botstein 1987), large multiplex families (Lathrop et al. 1984) identity by descent (Haseman and Elston 1972), inbred isolates (Guilford et al. 1994) and linkage disequilibrium (Friedman et al. 1994; Blanton et al. 1999).

The recognition of the value of deaf subjects from consanguineous marriages for homozygosity mapping has played a major role in the successful localization of many genes for recessive nonsyndromic deafness. Individuals of this type can be assumed to have inherited two copies of same recessive mutation carried by one of their common ancestors. The disease gene, along with closely linked markers are autozygous, or identical by descent. The mapping strategy involves typing the consanguineous deaf offspring and searching for chromosomal regions in which closely linked polymorphic markers are homozygous. Many of the markers now available are so polymorphic that it is unusual to observe homozygosity. In these circumstances, the observation of even a few deaf offspring of consanguineous marriages who are homozygous for alleles at the same locus may be sufficient to map the gene. When Lander and Botstein (1987) first called attention to the power of this mapping strategy, they advocated the use of isolated probands from consanguineous marriages and showed that as few as six to eight could be sufficient to map a locus. In practice, though, successful examples of homozygosity mapping have usually involved the analysis of multiple affected individuals in large consanguineous kindreds. However, the technique can be used successfully with isolated consanguineous probands, as was shown for biotinidase deficiency. Biotinidase deficiency is a recessively transmitted defect in the recycling of the vitamin

biotin. In the absence of normal biotinidase activity, affected individuals are entirely dependent on their dietary intake of biotin and typically begin to show symptoms of biotin deficiency in early infancy (Wolf et al. 1985). Hearing loss, which is eventually seen in about 60% of untreated patients, is a completely preventable complication of this disease. Subsequent to the introduction of newborn screening for this treatable form of genetic deafness, large numbers of affected individuals have been identified throughout the world. Table 4.1 shows the geotyping results for polymorphic markers in the p21–22 region of chromosome 3 in twelve isolated consanguineous probands with biotinidase deficiency, many of whom had been identified in newborn screening programs. The shaded area indicates the chromosomal regions in which the typed probands exhibited marker homozygosity. Initial analyses of eleven markers localized the gene to a small region containing D3S1286. Subsequent typing of probands P5 and P274 for six additional markers flanking D3S1286 further refined the critical region. Notice that, in contrast to inbred kindreds, the homozygosity involved different alleles in each proband. Proband P5 was the offspring of third cousins once removed and the conserved chromosomal region surrounding the biotinidase locus had been narrowed by recombination during a total of eleven meiotic divisions to a very small interval. By typing only twelve individuals, it was ultimately possible to assign the probable location of the biotinidase gene to the small segment of chromosome 3p between D3S3613 and D3S1286 (Blanton et al. 2000).

4. Functional Genomics of the Ear

As new genes for deafness have been mapped and cloned, the delineation of their base-pair sequences has frequently allowed their function to be surmised by matching the amino acid sequences of their protein products with data bases of genes whose function has already been established. This knowledge is providing dazzling insights into the normal and pathophysiology of hearing.

4.1 *Organogenesis*

Among genes that can cause deafness, some of the most exciting and potentially significant are those that encode DNA binding transcription factors. These genes produce proteins that bind to specific regulatory sequences on their target genes and act in concert with other transcription factors to promote or inhibit the activities of those genes (Avraham and Hasson, Chapter 2). The miraculous process of organogenesis that leads to the formation of a normal inner, middle and external ear results from a precise cascade of differential gene expression that is controlled by a hierarchy of DNA binding regulatory genes. The molecular defects in two forms of

TABLE 4.1. Homozygosity analysis in 12 consanguineous probands with profound biotinidase deficiency¹

Marker	Interval cM	Probands from the United States								Probands from Turkey				
		P3	P10	P7	P19	P21	P41	P16	P5	P274	P238	P241	P246	
D3S2387	16.6	193/205	173/197	185/197	181/197	193/205	193/197	185/205	201/205	–	193/197	197	177/197	
D3S1304	14.4	261/267	–	265	263/267	257/263	–	257/263	257/267	–	261	263	265/267	
D3S1259	2.7	196	206	204	200	204	200	204	196/200	200/204	196	196	196	
D3S1554 ^a	2.2	–	–	–	–	–	–	–	141	137/143	–	–	–	
D3S3510 ^a	0.0	–	–	–	–	–	–	–	179/183	283/289	–	–	–	
D3S3613 ^a	0.1	–	–	–	–	–	–	–	196	204/206	–	–	–	
D3S1286	0.0	143	143	147	147	143	119	143	137	141	119	145	143	
D3S3473 ^a	0.5	–	–	–	–	–	–	–	223	219	–	–	–	
D3S2338 ^a	0.5	–	–	–	–	–	–	–	179	191	–	–	–	
D3S3726	0.1	–	–	–	–	–	–	–	199/201	203	–	–	–	
D3S1293	5.7	122	–	140	122	130	122	130/142	130/136	132/140	122	130	142	
D3S2307	3.7	58	8	56	8	56	12/60	56/58	6	26/50	24/44	8	50	
D3S1266	10.4	296	301	301	296	291/295	289/297	297/295	293/295	–	295/299	293	293	
D3S1211	5.3	190	188	192	194	188	188/194	188/192	188	–	190/196	–	188	
D3S2432	3.0	146	150	146	146	150/154	146/150	150/154	138/154	–	130/146	126/150	146	
D3S1768	32.0	202	198	194	190	194/206	198/202	206	194/198	–	186/194	194	194	
D3S1285		238	238	234/238	232/234	238/240	–	236/238	232/238	–	234/238	232/238	238	

^a Eleven markers typed initially; five additional markers (*) flanking D3S1286 typed on probands P5 and P274 to further localize the gene.

genetic deafness have been shown to involve two members of the *POU* domain family of transcription factors (Griffith and Friedman, Chapter 6). This gene family was originally discovered in studies aimed at understanding how functionally distinct cell types arise within the pituitary gland. Many members of this family are expressed in fetal brain, including the neural tube and otic vesicle. De Kok et al. (1995) showed that the syndrome of X-linked congenital fixation of the stapes footplate with perilymphatic gusher (*DFN 3*) was caused by mutations in the *POU3F4* gene at Xq13. Mutations at this locus also lead to characteristic morphologic abnormalities in the internal auditory meatus, which can be detected by radio imaging and may contribute to the pathogenesis of the neurosensory component of the hearing loss. Mutations at a second locus *POU4F3* on 5q31 have been shown to be the cause of an autosomal-dominant form of hearing loss, *DFNA15* (Vahava et al. 1998). The expression of this gene is restricted almost exclusively to the fetal cochlea and knockouts of the murine homolog result in complete absence of the hair cells, with subsequent loss of the cochlear and vestibular ganglia cells. It seems likely that the gene must play some role in sustaining the hair cells, as well as initiating their differentiation in view of the progressive nature of the hearing loss in affected family members. *DFNA7*, a dominantly inherited form of progressive high-frequency hearing loss, was mapped to 1q21–q23 by Fagerheim et al. (1996). As noted by the authors, this region includes another member of the *POU* gene family, *POU2F1* that is also expressed in the embryonic cochlea of the rat.

4.2 Homeostasis

The high potassium concentration of the cochlear endolymph is a unique feature of the physiology of the ear, upon which health and normal function of the hair cells is dependent. An influx of potassium ions through gated potassium channels is required for transducing physical deflections of the hair cells into nerve impulses that can be processed and transmitted for subsequent neural processing. Several forms of genetic deafness now seem to have as a common denominator a defect in the maintenance of this critical potassium gradient. In the autosomal recessive Jervell and Lange-Nielsen syndrome, the defect involves one of at least two genes that code for proteins required to form normal potassium channels in the heart as well as the cochlea (Splawski et al. 1997; Duggal et al. 1998). In the heart, expression of the mutant genes leads to a characteristic prolongation of the QT interval, and a predisposition to syncopal attacks and sudden death. Heterozygotes for mutations at the *KVLQTI* locus on 11p15.2 and the *KCNE1* locus on 21q22.1 may exhibit the cardiovascular component of the syndrome without hearing loss. This dominantly transmitted phenotype, Ward-Romano syndrome, can also be caused by mutations involving other ion-channel genes (Wattanasirichaigoon and Beggs 1998). Whether

homozygosity at these loci will also lead to hearing loss remains to be determined.

The connexins are a somewhat similar class of genes, which code for the proteins that line the intercellular pores of gap junctions, where they facilitate the movement of small ions or molecules between cells. Defects in two members of this family, *Cx26* and *Cx31*, have been identified in patients with deafness (Kelsell et al. 1997; Xia et al. 1998). It is generally believed that, in the cochlea, the connexins facilitate the recycling of potassium ions from the hair cells back to the stria vascularis, where they can be actively transported back into the endolymph. If so, the hearing loss could result from an interference with this normal homeostatic mechanism.

Lastly, it has long been recognized that patients with *DFN3* have a mixed hearing loss with a significant sensorineural component in addition to the conductive loss resulting from their congenitally fixed stapes. It had always been assumed that the perilymphatic gusher, which is such a characteristic complication of surgical attempts to mobilize the stapes in this syndrome, must reflect an abnormal communication between the perilymphatic space and the epidural space of the CNS. Recent studies of the temporal bone by CT scans have shown a variety of developmental defects, including enlargement of the internal auditory canal and persistence of the vestibular aqueduct. It seems possible that an abnormal mixing of the high-potassium perilymph with the low-potassium cerebrospinal fluid may exceed the capacity of the stria vascularis to maintain the normal potassium concentration in the perilymph. If so, this mechanism could provide an explanation for the sensorineural component of the hearing loss.

4.3 Energy

Although the neurosensory structures of the organ of Corti are largely avascular, the stria vascularis is a highly vascularized structure, as its name suggests. This component of the cochlea is responsible for maintaining the endolymphatic potential. The high potassium concentration of the endolymph has been likened to a battery (Davis 1965), which stores energy by facilitating the flow of potassium ions across the stereocilia during sound transduction without requiring the active transport of the ions into the hair cell. Although the purpose of the battery is not known with certainty, by limiting the energy requirements of the hair cells it may increase the sensitivity of sound perception by allowing the neurosensory cells in the basilar membrane to function in a microenvironment that is devoid of turbulent blood flow.

In view of the energy requirements to sustain this system, perhaps it should have come as no surprise that genetic defects in the mitochondria are increasingly being recognized as potential causes for deafness (Fischel-Ghodsian, Chapter 7). Hearing loss can be a component of several syndromic forms of mitochondrial disease including MERRF and MELAS, but

there are three other mitochondrial mutations in which deafness plays a much more prominent role. One form of maturity onset diabetes of youth (MODY) has been identified which is caused by an A8334G substitution in the mitochondrial tRNA *Leu* gene (van den Ouweland et al. 1992). Most of these patients also develop a late-onset hearing loss, which can be quite rapid in its progression. Matrilineal transmission is a characteristic feature of these families. The A1555G substitution in the mitochondrial *12S* rRNA gene is now known to be the underlying cause for many cases of aminoglycoside ototoxicity (Fischel-Ghodsian et al. 1995). Aminoglycosides normally bind to bacterial rRNA molecules and exert their therapeutic effect by interfering with normal protein synthesis. The A1555G mutation makes the human mitochondrial ribosome more “bacteria-like” by creating a binding site for streptomycin, where it also interfaces with the fidelity of protein synthesis. The A1555G mutation has been shown to have a high prevalence in deaf populations in Mongolia, China, Japan, and Spain, and also appears to be the cause of hearing loss in some patients with no history of exposure to streptomycin. Whether there are natural compounds, or other toxins that can mimic aminoglycosides is not clear, nor is it clear why the effects of the gene are strictly limited to the ear, but at the same time expression can be so variable. Nuclear or mitochondrial modifier genes have been proposed as one possible explanation for the observed variability. Finally, an A7445G substitution immediately adjacent to the tRNA_{TRP} has been identified in several families with matrilineal hearing loss, in some of which ichthyosis was also found (Reid et al. 1994). The A7445G mutation interferes with the normal processing of the polycistronic message coded by the mitochondrial light chain. In a sample of 380 deaf students from Mongolia, loss of the *Xba* I restriction site was found in nine students, but sequencing revealed substitutions involving the 7444 and 7443 residues in addition to nt7445. Because these adjacent mutations all result in deafness, they may well define the binding site for the elusive endonuclease that initiates the processing of the light-strand message. Twelve of the students carried the A1555G substitution including, six who also had the 7444 change. Available clinical and audiologic data suggested that the individuals with the double mutations were more severely affected, raising the possibility of an epistatic interaction in subjects with the double mutant (Pandya et al. 1999).

4.4 Structure

Several genes for deafness code for structural proteins that appear to be required for normal hearing. The X-linked gene *Col4A* codes for a form of collagen that is deficient in Alport’s syndrome. In the kidney, this protein is an essential constituent of the basal membrane of the glomerulus. In its absence, the membrane becomes porous, allowing proteins and red cells to enter the glomerular filtrate. Although the physiologic role of the protein

in the cochlea is less well understood, it is known to be present in the basement membrane of the stria vascularis. Alpha tectorin is an important component of the tectorial membrane that supports the hair cells. Precisely how mutations in this gene cause deafness is not yet clear. In a Swedish family, Balciuniene (1998; 1999) found evidence for linkage of hearing loss to the alpha tectorin locus on 11q22 and also to a second locus, *DFNA2*, on chromosome 1p35.1. Subjects with both mutations had more severe hearing loss than those with single mutations at either locus, suggesting an epistatic interaction between the two loci. The C1057S substitution in the tectorin protein may have predisposed to abnormal crosslinking of the polypeptide.

5. Mating Structure of the Deaf Population

Assortative mating is a distinctive feature of the genetic architecture of deaf populations in many countries. One potent effect of this pattern of mate selection is to bring together rare genes at different loci that would otherwise have a low probability of coexisting in the same individual. Although 90% of deaf individuals in the United States marry deaf partners (Schein and Delk 1974), linguistic homogamy (shared manual communication), rather than phenotypic assortment for deafness, may in fact be the basis for mate selection. The observation suggesting this may be the case is the fact that the hearing partners in deaf-by-hearing matings are often “native signers” who are themselves the offspring of deaf couples. Despite the fact that they are not deaf, these individuals may carry genes for recessive deafness at multiple loci, and matings of this type can sometimes give rise to pseudodominant transmission of deafness.

5.1 Frequency of Common Forms of Deafness

The discovery that mutations in a single gene (*connexin 26*), are the commonest cause of genetic deafness was unexpected. Estimates of the relative frequency of *Cx26* deafness have varied greatly with up to 50% of all childhood deafness being attributed to this cause in some populations (Steel 1998). In others, such as India, Japan, Mongolia and China, the incidence appears to be much lower. Most reported studies have been based on molecular testing of clinic populations, and have not in general involved the random or stratified random sampling of subgroups likely to exhibit different frequencies. Adding to the confusion, probands with no affected siblings have in some reports been designated sporadic cases. The term sporadic refers to cases of deafness in which there is a very low chance of recurrence within the family, comparable to the incidence of deafness in the general population. Most sporadic cases of deafness are caused by environmental etiologies, but some can also represent new dominant mutations. Although probands with no affected siblings may be sporadic cases, they may also be isolated genetic cases in which by chance only one deaf child

has occurred in the family. One of the important goals of the genetic evaluation and counseling of the parents of deaf children has always been to try to identify the isolated genetic cases by clinical, genetic and (now) molecular criteria. Cases that were known a priori to be sporadic would not in general require molecular testing.

The existence of assortative mating among the deaf provides an alternative strategy for obtaining a robust estimate of the maximum frequency of Cx26 hearing loss in the deaf population. From the distribution of deaf and hearing offspring in DxD matings, segregation analysis permits estimation of the proportion of these marriages that can only have deaf children (non-complementary matings), the proportion that can only have hearing children (complementary matings), and the remaining proportion capable of producing both deaf and hearing children. The non-complementary matings reflect marriages between individuals who are homozygous for recessive alleles at the same locus, and can therefore only produce deaf offspring. The complementary matings include marriages between individuals with non-genetic deafness, nongenetic deafness and recessive deafness, or different types of recessive deafness. Finally, the segregating matings include offspring with dominant or pseudodominant phenotypes. In Table 4.2, some

TABLE 4.2. Statistics and Parameter Estimates from Segregation Analyses^a

<i>Fay Survey: Proband Matings</i>	
Number of fertile Deaf × Deaf matings	1,299
Total number of offspring	3,487
Nonsegregating sibships	0.831
All normal offspring (h)	0.789
All deaf offspring (y)	0.042
Segregating sibships (1-h-y)	0.169
Segregation ratio	0.325
<i>Fay Survey: Proband Sibships</i>	
Number of sibships	2,313
Total number of offspring	13,864
Proportion of genetic cases	54.9%
Proportion of dominants among genetic cases	12.0%
Segregation ratio for dominant cases	0.260
<i>National Survey (1970)</i>	
Total number of informative sibships	12,661
Total number of deaf offspring	16,482
Total number of offspring	49,765
Total number of deaf offspring	16,471
Sibships with deaf parents (DxD)	
Total number of sibships	421
Total number of children	1,356
Deaf children from non-complementary matings	451
Deaf children from segregating matings	538

^aTaken from Rose (1975)

of the parameter estimates that were observed by Rose (1975) in her segregation analysis of the Fay data are given. In that large, unselected, nationwide sample of 1,299 DxD matings, Rose estimated that only 4.2% were non-complementary. It seems reasonable to assume that these marriages were random with respect to the cause of deafness. If it is assumed that every case of non-complementation resulted from deaf parents who were both homozygous for mutations at the *Cx26* locus, the maximum possible frequency of the *Cx26* phenotype in the deaf population would simply be the square root of 0.042, or about 20.1%. Molecular testing in a small contemporary sample of 16 apparently non-complementary matings suggests that currently only 76% rather than 100% involve *Cx26* mutations, and there is reason to believe that fewer were attributable to this cause in the past. Thus, 17.8% would appear to be a conservative estimate of the maximum probable frequency of the *Cx26* phenotype in the deaf population during the 19th century.

Current estimates, using molecular testing, suggest that at least 36% of probands referred to clinics for evaluation of congenital sensorineural hearing loss have *Cx26* deafness, as do nearly 50% of probands from multiplex sibships (Green et al. 1999). It seems likely that the longstanding tradition of intermarriages among the deaf in this country is the explanation for the apparent doubling of the frequency of *Cx26* deafness during the past 100 to 200 years (Nance et al. 2000). It is reasonable to assume that in previous millennia the genetic fitness (i.e., relative fertility) of individuals with profound prelingual deafness must have been very low, perhaps approaching zero. Under those circumstances, virtually all new cases of deafness would have been born to hearing parents. During the last two centuries, the social, economic and educational circumstances of the deaf have begun to improve. As mentioned this trend has been accompanied by an increase in the fertility of the deaf, along with the onset of a substantial degree of assortative mating in many, but not all, populations.

It is widely recognized that, for continuously distributed genetic traits such as stature, the tendency for like to marry like has increased the variance or variability of the population beyond what it would be if marriages occurred at random with respect to stature (Fisher 1918). For a qualitative genetic trait such as deafness, the effect of assortative mating is to increase the frequency of the phenotype. In the limiting case, if deafness were determined by recessive genes at a single locus, and if all deaf individuals married one another, deafness would double in frequency in the first generation after the onset of assortative mating. The frequency would then continue to increase until the incidence of deaf homozygotes began to approach the gene frequency in the population. This is the potential consequence of continued intermarriage among the deaf, which was of concern to A.G. Bell (Bell 1883).

Because of the extreme degree of genetic heterogeneity known to be associated with deafness, it has always been assumed that any effect of assorta-

tive mating on the frequency of deafness would be statistically trivial. The discovery that one form of recessive deafness is so much more common than all others, raises the possibility that this assumption may not be correct. In each generation after the appearance of assortative mating, the deaf children of deaf parents entered the new deaf-by-deaf mating pool, along with a substantially constant frequency of deaf offspring with genetic deafness who were born to hearing parents. However, the relative frequency of genes for different forms of recessive deafness should not be the same in the two groups. Among the former, many of the deaf offspring are the products of noncomplementary matings. Since the frequency of non-complementary matings for each type of recessive deafness is proportional to the fourth power of the respective gene frequencies, there is a strong bias towards the transmission of the most common form(s) of recessive deafness to the deaf offspring from these matings. The net effect of this process is the preferential transmission of *Cx26* deafness to the deaf-by-deaf mating pool of the succeeding generation. This in turn will progressively increase the frequency of *Cx26* deafness, the proportion of non-complementary matings, and the overall incidence of genetic deafness. Nonrandom mating by itself can only alter the genotype frequencies and not the underlying gene frequencies. But, when it is accompanied by relaxed selection, it can greatly accelerate the changes in gene frequency that can accompany attainment of a new mutational equilibrium. The magnitude of these effects will depend on many factors, including the number of recessive forms of deafness and their relative frequency, the overall proportion of deafness that is genetic, and the relative frequency and fertility of marriages among the deaf.

For any genetic trait, it is to be expected that gene and genotype frequencies vary in different populations; but in the case of the genes that cause deafness, the mating structure of the population is another important potential source of variation. Available data suggest that the incidence of *Cx26* deafness in India (Green, personal communication), Mongolia (Pandya, personal communication), China (Liu, personal communication) and Japan (Fuse et al. 1999) is substantially lower than in populations where there has been a long tradition of intermarriages among the deaf. In the past, marriages among the deaf were virtually unheard of in India. In Mongolia, not a single one of 380 probands studied at the School for the Deaf in Ulan Bator was the offspring of a DxD mating. In China, Liu observed only two DxD matings among 184 marriages of the deaf (Liu et al. 1994). On the other hand, if there are populations in which the total proportion of *Cx26* deafness is 50%, for example, then 25% of all marriages among the deaf should have all deaf offspring because of non-complementation. In the limiting case, if all deafness in a population is caused by a recessive mutation at a single locus, 100% of marriages among the deaf should be non-complementary. This finding appears to characterize the genetic epidemiology of *DFNB3* in the Balinese population reported by Friedman et al. (1995).

5.2 *Pseudodominance*

Knowledge of the high frequency of *Cx26* deafness provides a satisfying explanation for two anomalies in Rose's analysis of the Fay data set (Table 4.2). In the proband sibships, which included data on the phenotypes of parents and siblings obtained by selection through affected probands, Rose estimated that the proportion of dominant phenotypes among the genetic cases was 12%. In contrast, the proband matings that were ascertained by selection through affected parents yielded an estimate of 16.9% for the frequency of segregating matings, which were assumed to result primarily from dominant transmission of the deafness phenotype. Furthermore, the estimated segregation ratio for the dominant cases in the proband sibships was 0.26, while the ratio in the segregating proband matings was 0.325. In view of the low penetrance for deafness in dominant phenotypes such as Waardenburg syndrome, the observation of a low segregation ratio for dominant deafness was not surprising. However, there would seem to be no good explanation why the penetrance should vary with the mating type of the parents. It now seems likely that the increased frequency and penetrance of the deafness phenotype in the segregating DxD matings reflects the presence of fully penetrant pseudodominant *Cx26* phenotypes in about 19% of the segregating DxD matings. Over time, the frequency of pseudodominant transmission would be expected to increase in an assortatively mating population as the frequency of gametes carrying multiple genes for deafness increases.

Evolutionary biologists now agree that the acquisition of syntactic speech 50,000 years ago resulted in an explosive acceleration in the evolution of the human brain.

The recent cloning of a major gene that is of importance for the development of speech (Lai et al. 2001), suggests that this process may have been initiated by the mutation and subsequent fixation in the population of a relatively small number of genes. The rapid changes that have occurred in the frequency of *Cx26* deafness following the introduction of sign language suggest that the combination of linguistic homogamy and improved genetic fitness may also have contributed to the rapid evolution of speech. This mechanism will also amplify the frequency of genes that interact with the primary locus and could therefore have contributed to selection for genes that influence other mental traits that depend upon speech and language for their expression. In this regard, it is of great interest that epistatic interactions with mutations at separate loci are now being recognized to be an important cause for "digenic" deafness in deaf subjects who carry only a single pathologic mutation at the *Cx26* locus (Lerer et al. 2001).

5.3 *Potential Effects of Marital Selection for Cx26*

Some deaf couples do not regard hearing loss as a handicap and would prefer to have deaf children. Most express no strong preference, while many would clearly prefer hearing children (Middleton et al. 1998). Since testing

for Cx26 deafness is becoming more widely available in the deaf community, it is useful to assess the range of the potential effects that positive or negative marital selection for Cx26 genotypes might have on the overall incidence of deafness. Using data on the overall frequency of deaf children who are born to deaf parents (Table 4.2) and data on the frequency of pseudodominance and Cx26 deafness, it can be estimated that the complete avoidance of at-risk Cx26 marriages among the deaf would lead to approximately a 2% reduction in the overall incidence of deafness. Conversely, complete genotypic assortment for Cx26 deafness would lead to approximately an 8% increase in the first generation. The long-term effect of continued complete genotypic assortment would be the progressive reduction of heterozygotes in the population until the frequency of homozygotes began to approach the gene frequency of about 1.5% (Green et al. 1999). In comparison with phenotypic assortative mating, genotypic mate selection would greatly accelerate the approach to this limit. Nevertheless, despite the extreme nature of the alternative assumptions, the immediate effects of genotypic mate selection are relatively modest.

5.4 Estimating the Frequency of Common Forms of Deafness

Clearly, the frequency of a common form of hearing loss such as Cx26 deafness can differ among racial or ethnic groups. Since it is a genetic form of deafness, the incidence will also differ in probands from simplex and multiplex sibships; and because of the mating structure of the deaf population, the incidence will even differ in the deaf offspring of deaf and hearing couples. For these reasons, obtaining reliable estimates of gene and genotype frequencies will require a random sample that includes proportionate representation from all relevant subgroups of the deaf population, or at least knowledge of the distribution of these subgroups, so that overall estimates can be reconstructed from stratified samples.

5.5 Other Examples of Nonrandom Mating

Phenotypic assortative mating is not the only form of nonrandom mating that can alter the frequency of genotypes from those expected under Hardy-Weinberg equilibrium. Inbreeding increases the frequency of all rare recessive phenotypes above the values expected with random mating. Similarly, were it not for racial and ethnic homogamy, the frequency of sickle cell anemia and Tay-Sachs disease (at conception) in this country would be far lower than the current incidence. These two diseases are useful examples for those who might be alarmed by the increase in the incidence of Cx26 deafness that appears to have occurred in this country during the past century. Marriages among the deaf are an integral part of a culture that has greatly enriched the lives of both the deaf and hearing segments of society during the past two centuries. Unless we are prepared to advocate the

prohibition of racial and ethnic homogamy, there would appear to be no rational justification for deploring the effect that assortative mating may have had on the incidence of genetic deafness.

6. Summary

Interest in the genetics of deafness has a long history that predates the rediscovery of Mendelism. Throughout most of the twentieth century, geneticists argued about whether the genetics of deafness could best be explained by dominant or recessive genes at one, two, three, or perhaps four loci. By the 1970s, the concept of etiologic heterogeneity was well established. However, it seemed inconceivable that it would ever be possible to isolate and purify sufficient quantities of specific proteins from the cochlea to actually identify the functions of the mutant genes for the various forms of syndromic deafness that were being recognized. This volume is a testament to the revolutionary impact that molecular genetics has had on this field since that time. A unique feature of the genetic epidemiology of deafness is the cultural variation that exists in the mating structure of the deaf population. Now that the prevalence of specific genes in a population can be measured, we are beginning to appreciate the profound effect that recent changes in the mating structure have had on the frequency and distribution of genes for deafness.

One of the major limitations of man as the object of genetic research is the inability to *perform* experimental matings. As molecular testing for specific forms of genetic deafness becomes available, the existence of assortative mating among the deaf will ultimately provide an unparalleled opportunity to search for interactions among non-allelic genes for deafness. Since the conservation of phenotypes across species is far less complete than the conservation of orthologous gene sequences, phenotypes that result from interactions among genes are likely to be even less completely conserved. Human model systems will therefore be essential to recognize these effects. It should already be possible to ask, for example, if heterozygosity for a connexin 26 mutation alters the expression of Waardenburg syndrome, Pendred's syndrome, the branchio-oto-renal syndrome, or Jervell and Lange-Nielsen syndrome. The work of Morell et al. (1997) provides hints on possible interactions between the WS 2 (*MITF*) and ocular albinism (*TYR*) genes, and the observations of Balciuniene et al. (1998, 1999) suggest apparent interactions between genes at the *DFNA2* and the alpha tectorin locus. Thus, specific digenic interactions may be an important cause for variation in expressivity. However, as more deaf people get cochlear implants, the opportunity to document the effects of gene interactions on phenotype is likely to begin to disappear.

The knowledge that some forms of genetic deafness such as *connexin 26* and A1555G are much more common than all other types in some populations has also made it possible to contemplate radical "postgenomic" strate-

gies for identifying new genes for deafness. With the successful completion of the mapping of the human genome, assigning functions to the genes that have been mapped has emerged as a major goal of research in human genetics. It may no longer be necessary to engage in the laborious collection and genotyping of samples from large pedigrees, consanguineous families or even sib pairs. By implementing a sequential screening strategy, beginning with the forms of deafness that are most common and easiest to test for, it may prove feasible to simply screen large repositories of DNA samples from probands in multiplex sibships for mutations in plausible candidate genes and/or murine orthologs by direct sequencing or other techniques. In this way, it may ultimately be possible to determine the frequencies of genes for deafness in different racial and ethnic groups, as well as the distribution of mutations in these genes.

Lastly, progress will undoubtedly be made in specific therapies to treat or prevent hearing loss. Biotinidase deficiency and streptomycin ototoxicity are perhaps the only forms of genetic hearing loss for which preventive pharmacologic treatment is already available, by providing supplemental biotin in the first instance, and avoiding aminoglycosides in the second. Dramatic though the results of cochlear implants have been, this therapy may some day be supplanted by the use of genetically corrected autologous stem cells to replace defective hair cells, or regenerate other specific cellular components of the cochlea. If one or more of these therapeutic approaches are successful, the 20th century may well have been the last in which deafness was a familiar part of the human experience.

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5

Cytogenetics and Cochlear Expressed Sequence Tags (ESTs) for Identification of Genes Involved in Hearing and Deafness

ANNE B. SKVORAK GIER SCH and CYNTHIA C. MORTON

1. Introduction

A variety of approaches is being used to study the genetics of mammalian hearing. Most methods have the same goal: to first identify the chromosomal location of genes that contribute to hearing loss, and then to discover the gene itself. This chapter addresses two methodologies that can be employed in gene discovery in the auditory system: cytogenetics and cochlear expressed sequence tags. Cytogenetics is the study of chromosomes. Structural rearrangements of chromosomes, such as deletions or translocations, may be associated with disease. These rearrangements can cause disruption or deletion of a particular gene(s), thus detection of a chromosomal rearrangement can lend clues to where a disease gene resides. Cochlear expressed sequence tags (ESTs) offer a glimpse at gene expression in the sensory end organ for hearing. Sequence analysis of ESTs provides a survey of genes expressed in the cochlea and a collection of positional candidate transcripts for deafness loci.

2. Cytogenetics

Eukaryotic chromatin, composed largely of DNA, histones and nonhistone proteins, is in a decondensed form in the nucleus throughout the majority of the cell cycle. However, during the relatively brief mitotic stage of the cycle, M phase, newly replicated DNA condenses into discrete units, or chromosomes, in preparation for cell division. Cytogenetic studies are concerned primarily with the chromosomes in mitosis, when each of the individual chromosomes can be visualized simultaneously as a separate, subnuclear component using the light microscope.

Tjio and Levan (1956) were the first to determine that the correct number of chromosomes per human somatic cell is 46. Human chromosomes were grouped according to size and centromere position, but were not individually identifiable until the 1970s, when a variety of banding techniques was

introduced (Caspersson et al. 1970; Drets and Shaw 1971). The most common technique used today, G-banding, is shown in Figure 5.1. Each of the chromosomes can be identified by its size, shape, and characteristic banding pattern. The light and dark bands of each chromosome are numbered according to an accepted international standard (Fig. 5.2) (Mitelman 1995). By convention, the chromosomes are ordered essentially from largest to smallest, with the shorter arm of each chromosome (the p arm) positioned on top, and the longer q arm below.

When a chromosomal rearrangement such as a translocation or deletion is described, it is done according to nomenclature guidelines that cite the chromosome(s) involved and the band where the break is thought to have occurred (Mitelman 1995). For example, a deletion (del) of chromosomal material from the long arm of chromosome 14 between bands q22 and q23 is denoted as del(14)(q22q23). A translocation (t) between the short arm of chromosome 3 from band p24 to the end of the short arm (pter), and the long arm of chromosome 8 from band q13 to the end of the long arm (qter), is described as t(3;8)(p24;q13).

2.1 *Cytogenetic Causes of Human Disease*

Over the course of hundreds of thousands of years of evolution, the human species has come to maintain a relatively stable karyotype. Gross variation in the number or structure of human chromosomes severely reduces genetic fitness. It is estimated that the human complement of 46 chromosomes contains 50,000 to 100,000 genes, with temporal and spatial regulation of each. With few exceptions, addition or deletion of whole chromosomes (aneuploidy) is incompatible with life. Large subchromosomal deletions or duplications are similarly lethal, whereas fetuses with small deletions or duplications may be viable. A large percentage of the DNA that comprises human chromosomes does not encode proteins, but even small, submicroscopic pieces of chromosomes can contain dozens or hundreds of genes. Chromosomal rearrangements such as translocations can abrogate gene expression, resulting in multiple congenital anomalies, similar in nature to an autosomal dominant mutation.

2.1.1 Aneuploidy

The first chromosomal disorders reported to cause human pathology were aneuploidies. Lejeune et al. reported in 1959 that nine children with “mongolism” (Down syndrome, Fig. 5.3) had an additional small chromosome (now known to be chromosome 21) (Lejeune et al. 1959). In the same year, a number of sex chromosome aneuploidies were reported, including Turner syndrome (45,X, Fig. 5.4), in which a female typically has only 45 chromosomes, with monosomy X (Ford et al. 1959), and Klinefelter syndrome (47,XXY), in which a phenotypic male has two X chromosomes and one Y,

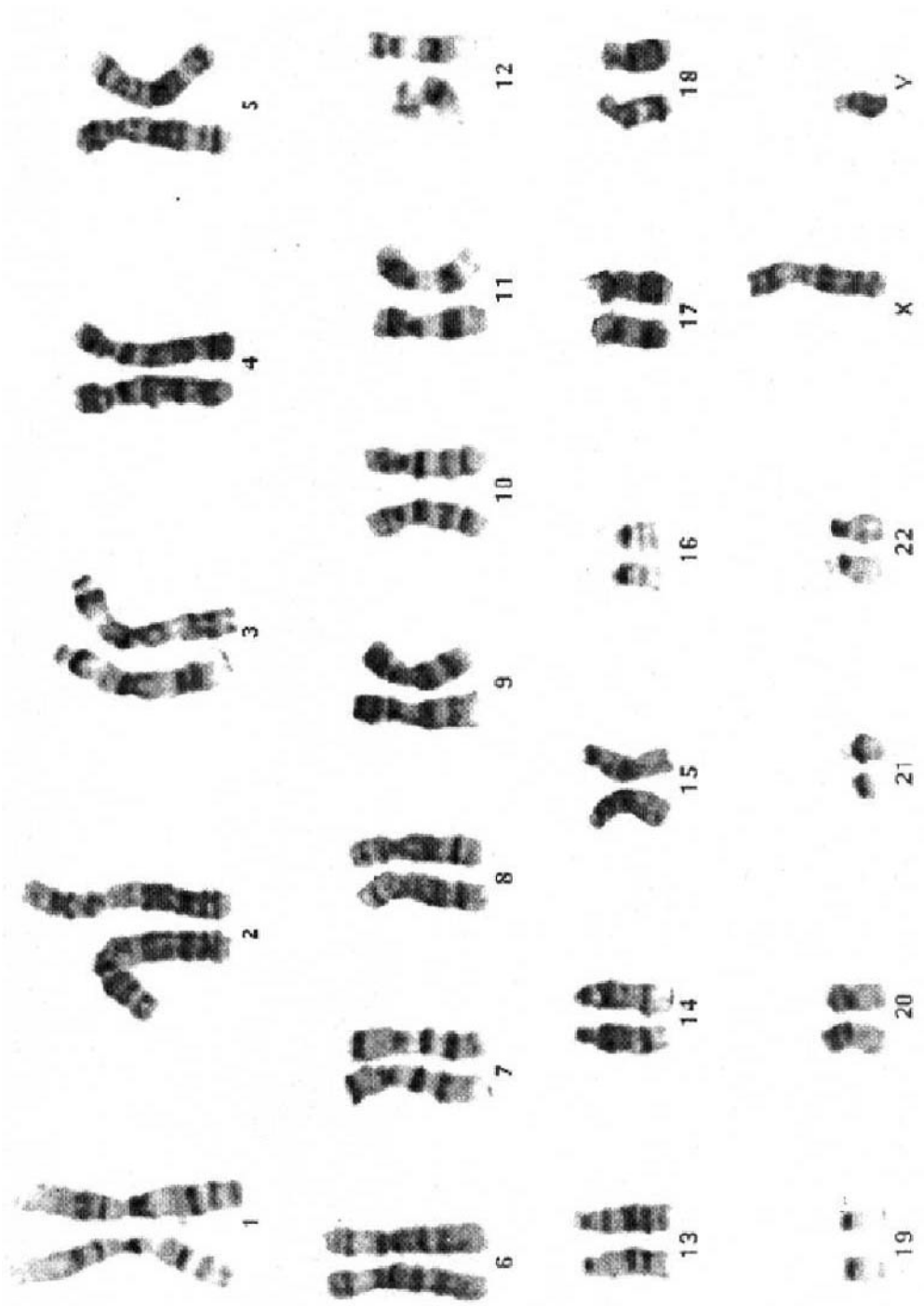


FIGURE 5.1. G-banded human male karyotype (46,XY), consisting of the normal complement of 46 chromosomes, including one X and one Y.

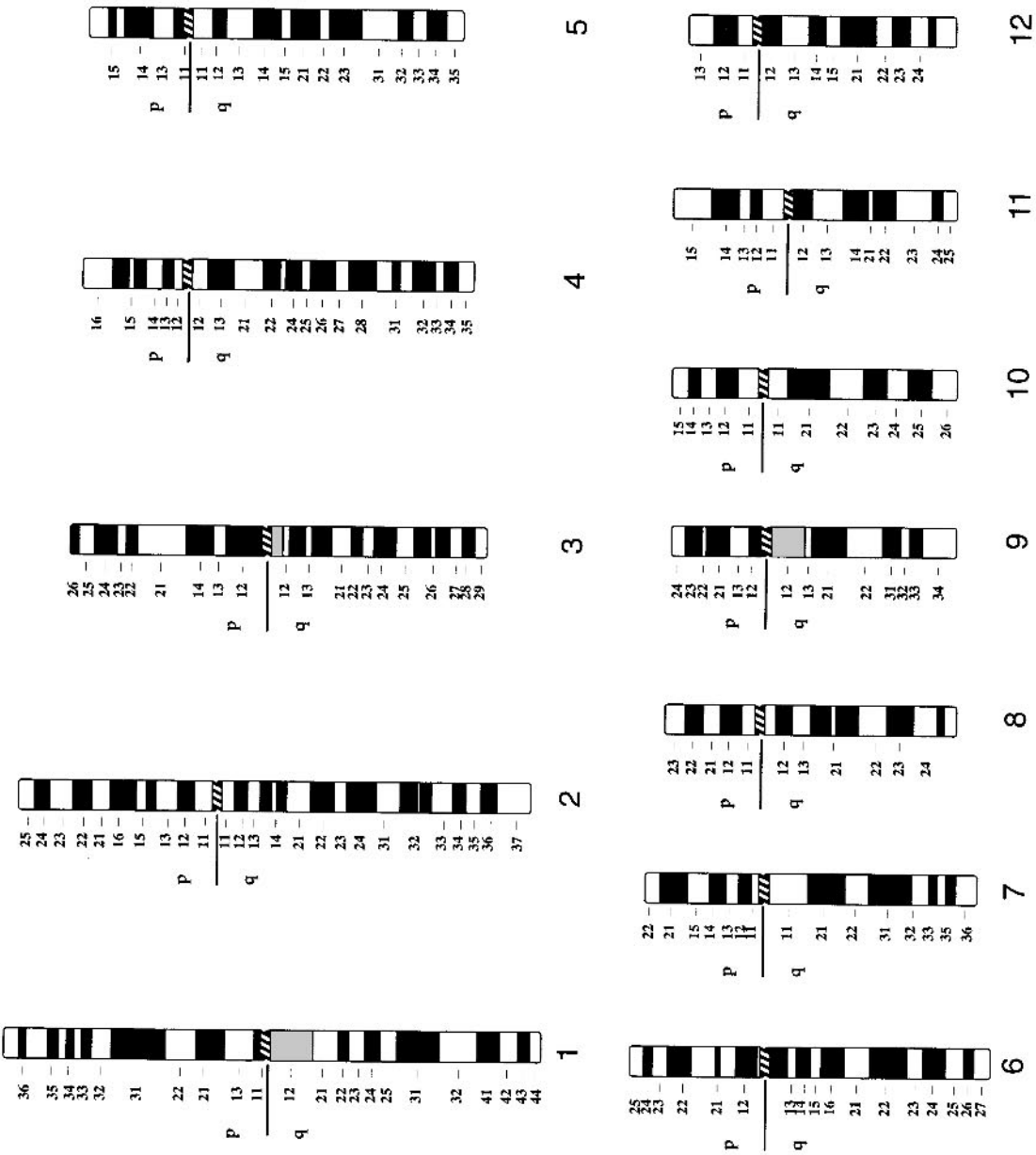
for a complement number of 47 instead of the usual 46 (Jacobs and Strong 1959). Additional sex chromosome aneuploidies, such as 47,XXX, 47,XYY and other combinations, were also described.

Of all cytogenetic abnormalities observed, aneuploidies are the most common. They account for greater than 90% of the chromosomally abnormal newborns and spontaneous pregnancy losses (Therman and Susman 1993). The sex chromosome aneuploidies are the only variations in total chromosome number that can yield a relatively healthy individual. Indeed, often patients with sex chromosome aneuploidies are ascertained only at puberty or in adulthood when sexual developmental or reproductive difficulties arise. Among the autosomal trisomies, only trisomy 21 is compatible with a relatively long life, but it is characterized by multiple developmental problems, both physical and mental. Trisomy 21 is the single most common cause of mental retardation, and has the highest incidence of any autosomal chromosomal aneuploidy in liveborns (Gardner and Sutherland 1996). Other autosomal trisomies, such as trisomies 13 and 18, are seen among liveborns, but these infants have severe mental and physical handicaps, and rarely survive for more than a year (Therman and Susman 1993). If these trisomies are found in a mosaic state, where only a subset of cells in the body has the abnormal number of chromosomes, the individual may live many years, though a wide spectrum of disabilities, from very mild to profound will typically be present. Other trisomies or monosomies are generally only seen in stillbirths or miscarriages, reflecting the severity of the chromosomal imbalance on fetal development.

2.1.2 Unique Chromosomal Rearrangements

A variety of chromosomal rearrangements is possible, having been seen in human karyotypes. A translocation is an exchange of genetic material between two chromosomes. Translocations can be either balanced or unbalanced. The term balanced implies an exact exchange of chromosomal material. Constitutional balanced translocations are usually without clinical significance to an individual. Approximately 1 in 500 newborns are balanced translocation carriers (Hook and Hammerton 1997). However, an apparently balanced translocation can also cause gene disruptions or fusions, resulting in an untoward outcome. For example, studies show that balanced translocations are five times more frequent in mentally retarded individuals than in the general population (Funderburk et al. 1977).

The best studied translocations are the acquired translocations found in various cancers, especially hematological disorders. Perhaps the most well known translocation in human disease is the "Philadelphia" chromosome described by Nowell and Hungerford in leukemic cells from patients with chronic myeloid leukemia (Nowell and Hungerford 1960). Named for the city in which it was discovered, the Philadelphia chromosome results from a balanced translocation between chromosomes 9 and 22, t(9;22)(q34;q11).



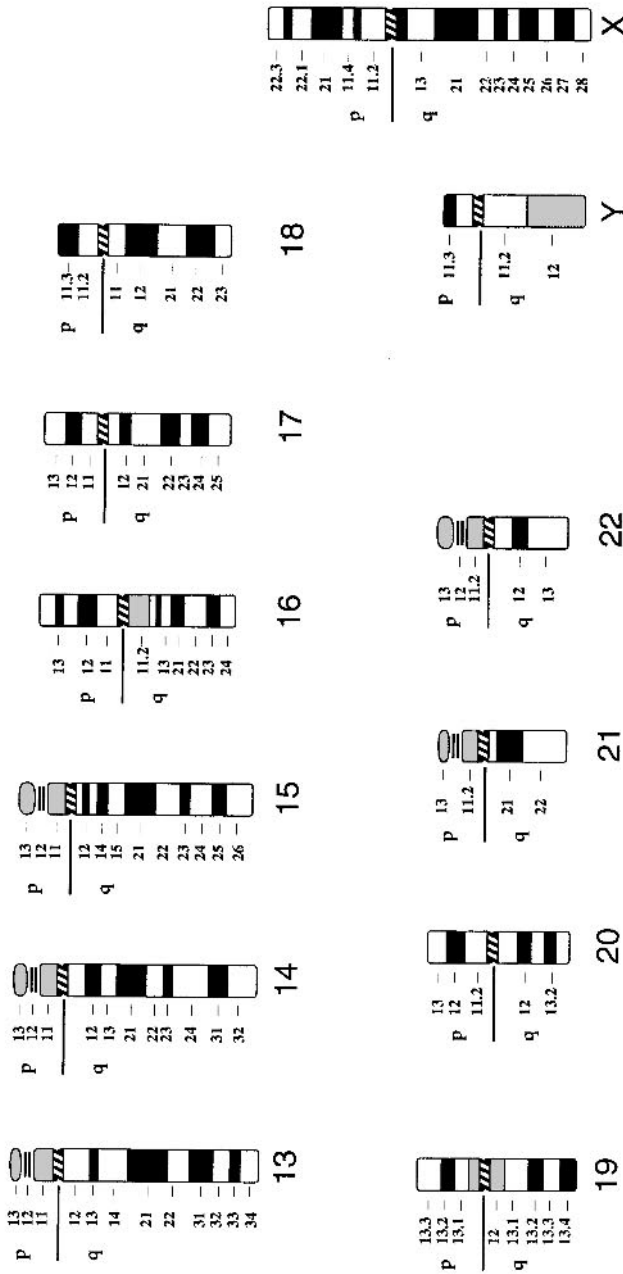


FIGURE 5.2. Ideograms of the 24 human chromosomes. Black and white bands correspond to G dark and light bands, respectively. Shaded bands represent heterochromatic regions. Hatched areas mark the centromere positions. Bands are numbered according to the International Standard of Human Cytogenetic Nomenclature (Mitelman 1995).

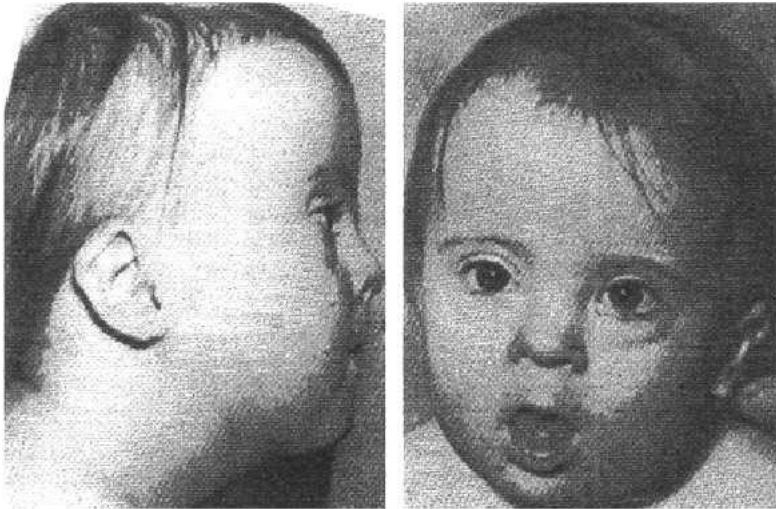


FIGURE 5.3. Infant with Down syndrome. Notice low-set, posteriorly rotated ears, flat facial profile and protruding tongue (Jones 1997, with permission of W.B. Saunders Co.).

It is detected in approximately 95% of CML patients. The translocation creates a fusion transcript between the *BCR* (break point cluster region) gene on chromosome 22 and the *ABL* (Abelson) gene on chromosome 9. The fusion protein is thought to create an aberrantly regulated kinase that activates a number of signal transduction proteins, leading to dysregulated cellular proliferation.

Inversions are due to breakage and reunion within the same chromosome. Inversions are of two types: pericentric, which involves a break in each arm with the centromere in between, and paracentric, in which both breaks are within the same chromosomal arm. A number of inversions, such as a small pericentric inversion involving chromosome 9, appear to be clinically insignificant, and are recognized as a chromosome polymorphism in humans. About one percent of the human population carries a chromosomal inversion without phenotypic consequence (Therman and Susman 1993). However, inversions can cause disease when a breakpoint occurs in a functional gene. Difficulty can also arise during meiosis when an inverted chromosome attempts to pair with its normal homolog. Depending on the size of the inverted region, faulty pairing and missegregation can occur, resulting in deleted or duplicated chromosomal segments.

2.1.3 Deletion Syndromes

Chromosomal deletions are usually associated with a constellation of clinical findings. A number of well known chromosomal deletion and

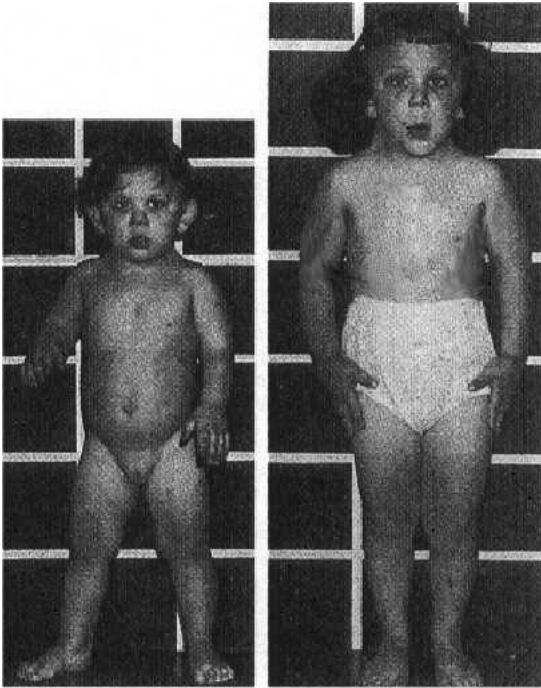


FIGURE 5.4. Girl with Turner syndrome at ages 2 and 4 years. Notice large, prominent ears, webbed neck, and short stature (Jones 1997, with permission of W.B. Saunders Co.).

microdeletion syndromes exist that give rise to recognizable genetic disorders. Among these are the DiGeorge/velocardiofacial syndromes, which usually result from a deletion in chromosome 22 in band q11, and the Prader-Willi/Angelman syndromes, which are most often caused by deletions of chromosome 15 in bands q11-q13. Deletions in some disorders are large enough to be seen on a routine karyotype, but molecular techniques such as fluorescence in situ hybridization (FISH), where fluorescently labeled DNA probes are hybridized to chromosome spreads, have become the standard for diagnosis of these disorders.

The clinical phenotype resulting from a chromosomal deletion can be due to loss of one critical gene, or to a set of contiguous genes. For example, Alagille syndrome is characterized by ocular, skeletal and cardiac defects in association with loss of intrahepatic bile ducts. Additional anomalies may also be present. A large deletion of chromosome 20p12 is observed in several families with Alagille syndrome; however, point mutations in the gene *Jagged1* (*JAG1*), which maps to this locus, also cause the same syndrome (Li et al. 1997; Oda et al. 1997), suggesting deletion of only this one gene is sufficient to cause the syndrome. In contrast, Miller-Dieker syn-

drome is caused by the deletion of multiple genes. Miller-Dieker patients have lissencephaly, severe mental retardation, and a characteristic facial appearance. They may also have growth retardation, heart defects, and seizures (Dobyns et al. 1991). Ninety percent of Miller-Dieker patients have deletions in chromosome 17p13. More than one gene must be involved in the syndrome because mutations in the *LIS1* gene at this locus cause isolated lissencephaly, without the other features characteristic of Miller-Dieker syndrome (Chong et al. 1997). Presumably, deletion of genes in addition to *LIS1* at 17p13 contribute to the full spectrum of anomalies.

Miller-Dieker syndrome illustrates how analysis of individual genes within a critical deletion region can help establish which gene is responsible for a specific feature of a syndrome. Collections of overlapping deletions are used to narrow the critical region, allowing identification and analysis of individual genes and the role they play in the pathobiology of the disease.

Alternatively, chromosomal deletions may cause disease by unmasking recessive alleles on the sister chromosome. This mechanism may be the etiology for the hearing loss associated with Smith-Magenis syndrome (Greenberg et al. 1996).

2.2 *Cytogenetics and Hearing Loss*

Hearing loss is a component of a number of chromosomal syndromes. Aneuploidies, translocations, inversions, duplications, and deletions have each been found that cause hearing loss. However, cytogenetics has not been a traditional technique used in investigations of genetic deafness. No large scale cytogenetic studies of patients with isolated hearing loss have been reported.

In the next sections, some of the syndromes and isolated cases of chromosomal rearrangement in which hearing loss has been found are reviewed. Cytogenetic studies may complement molecular investigations, allowing a better understanding of a syndrome and the gene(s) that cause it. Three specific examples in which cytogenetic findings facilitated gene discovery are discussed below.

2.2.1 Aneuploidy and Hearing Loss

Depending on the missing or additional chromosome, individuals with chromosome aneuploidies can have a wide spectrum of physical and mental handicaps, reflecting developmental disorders at various stages of fetal life. Generally, the more complex organ systems or structures appear to be affected most profoundly. Thus, among autosomal aneuploidies, the brain is uniformly abnormal, and physical anomalies, especially craniofacial, are typical. Heart, genitourinary system, eyes, hands and feet are also often involved (Therman and Susman 1993). It is of no surprise that hearing loss or deafness is found in several of the human aneuploidies.

Studying hearing loss in aneuploidies does not lend itself easily to a better understanding of hearing and deafness in general. Because of the wide spectrum of malformations in most patients with chromosomal aneuploidies, and the various stages of development that are affected, it is difficult to determine which genes on the extra or missing chromosome influence the normal development or function of any particular organ system. However, because of the prevalence of hearing loss in individuals with aneuploidy syndromes, it represents a significant aspect of health care management of such patients. Undiagnosed hearing loss adds another invisible burden for individuals who are already challenged with mental retardation or physical difficulties.

2.2.1.1 Trisomy 21 and Hearing Loss

Down syndrome occurs in about 1 in 700 newborns (Fig. 5.3). Individuals with Down syndrome have a host of clinical findings, including mental retardation, short stature, hypotonia, characteristic facial features, cataracts, heart defects, thyroid disorders, an increased incidence of leukemia, and premature aging (Jones 1997). Hearing loss is found in about 40% to 80% of patients, depending on the threshold level used and method of testing (Roizen et al. 1993). The hearing loss is generally mild, bilateral conductive; however, sensorineural hearing loss was detected in 34% of children evaluated in one study (Roizen et al. 1993). The external ears of Down syndrome patients tend to be simple and low set with an overfolded upper helix and small or absent lobes (Jones 1997). Children tend to have dysfunctional Eustachian tubes, leading to a high incidence of otitis media and accounting for some of the conductive hearing loss (Roizen et al. 1993). The pathogenesis of the sensorineural hearing loss in individuals with Down syndrome has not been determined, although a gene dosage effect of a gene on chromosome 21 must be considered a likely mechanism.

2.2.1.2 Trisomy 13 and Hearing Loss

With an incidence of 1 in 12,000 (Hook 1980), trisomy 13 is much rarer in liveborns than is trisomy 21, and the phenotype is much more severe. Ninety percent of trisomy 13 infants die before 6 months of age. The infants are severely retarded due to various types of forebrain defects. More than 50% of cases are noted to have eye defects ranging from anophthalmia to microphthalmia, cleft lip and/or cleft palate, polydactyly, microcephaly, heart defects, renal anomalies, and deafness (Jones 1997).

Individuals with trisomy 13 mosaicism may show a less severe phenotype than those with a full trisomy. Presumably, the presence of a normal cell line, especially in particular tissues and organs, mitigates the effects of the trisomic cells. There have been at least two reports of hearing loss or deafness in patients with mosaic trisomy 13 (Delatycki and Gardner 1997), but the mechanism of this pathology remains unclear.

2.2.1.3 *Turner Syndrome and Hearing Loss*

Turner syndrome, as illustrated in Figure 5.4, is characterized by monosomy X (45,X) in 50% of cases and various mosaicisms or structural abnormalities of the other X chromosome in the remaining cases. The incidence is about 1 in 2,500 live births, but the frequency at conception is much higher. Greater than 95% of 45,X concepti result in spontaneous loss (Gardner and Sutherland 1996). Females with Turner syndrome are characterized by small stature, streak gonads, short or webbed neck, broad chest with widely spaced nipples, prominent ears, epicanthal folds, and a high-arched palate. Coarctation of the aorta and renal anomalies are common findings. Intelligence is usually within normal limits (Jones 1997). About half of Turner syndrome females have moderate sensorineural hearing loss, often combined with conductive hearing loss. Chronic or recurrent ear infections are common in childhood and may account for some of the conductive hearing loss (Gorlin et al. 1995). The nature of the sensorineural hearing loss is unknown.

2.2.2 Unique Chromosomal Rearrangements Associated with Hearing Loss

There are a number of reports in the literature of various chromosomal rearrangements and hearing loss. Unlike aneuploidies and microdeletion syndromes, these are not disorders that have been seen repeatedly. Instead, they are rearrangements that are either unique, or reported in only a few individuals. In each case, the chromosomal rearrangement was discovered either at amniocentesis, or in the evaluation of a dysmorphic, developmentally delayed child. Hearing loss is usually just one finding of many physical abnormalities. All the children have various degrees of mental retardation and dysmorphic features. Hearing loss is probably underdiagnosed among infants born with unusual chromosomal rearrangements, but given the severity of the physical problems present at birth a hearing test may not be considered a high priority.

2.2.2.1 *An Unusual Marker Chromosome 15*

Marker chromosomes are chromosomes that are structurally abnormal. They are often difficult to identify, and specific tests are required to determine the origin of the chromosomal material. The prevalence of marker chromosomes is less than 0.7 per 1,000 births (Gardner and Sutherland 1996). An inverted duplication of chromosome 15 is among the more common markers. Generally, the centromere and the proximal portion of the q arm are duplicated and inverted. Small inverted duplications may have no phenotypic effect, whereas larger ones produce characteristic mental retardation and dysmorphic features (Gardner and Sutherland 1996). Hearing loss among individuals with the “common” inverted duplication of chromosome 15 is not a typical finding. However, Huang et al.

(1998) reported an unusual inverted duplication of chromosome 15 involving the distal portion of chromosome 15q, rather than the proximal, from q25 to qter (Fig. 5.5A). The infant had severe hypotonia, cardiovascular defects, CNS anomalies, and dysmorphic facies. Severe hearing loss was also present, as determined by auditory evoked brainstem response. The child had such severe developmental anomalies that she died at 12 days of life.

2.2.2.2 *Inversion 2*

Pericentric inversions are the most frequent chromosomal rearrangements in humans, occurring in approximately 1% of the population (Therman and Susman 1993). The majority are inherited and clinically insignificant. Pericentromeric inversions of chromosome 2 are the second most commonly recognized inversion of human chromosomes, after pericentromeric inversion of chromosome 9. Usually, the breakpoints are in p11q13. Kozma et al. (1996) reported an unusual inversion of chromosome 2, with breakpoints in p13q11.2 (Fig. 5.5B). The child had craniofacial anomalies, significant hypotonia, developmental delay, and severe to profound bilateral hearing loss.

2.2.2.3 *Partial Trisomy 6q*

Conrad et al. (1998) reported a toddler with a partial trisomy of approximately the lower third of chromosome 6 (Fig. 5.5C). The child had microcephaly, facial anomalies, a webbed neck, congenital heart disease, renal hypoplasia, developmental delay, and bilateral hearing loss. The additional portion of chromosome 6 was translocated to the short arm of chromosome 14 and was inherited from the child's father, who had an apparently balanced translocation between 6q22 and 14p13.

2.2.2.4 *A Tandem Duplication and Deletion*

Meschede et al. (1998) reported a translocation between two acrocentric chromosomes, 14 and 21, with essentially the entire chromosome 21 translocated to the telomeric end of 14q, resulting in a small deletion of 14q32.3 (Fig. 5.6). The phenotype included developmental delay, severe hypotonia, mild facial dysmorphism, growth retardation, hypospadias, palmar creases, marbled skin and a patent ductus arteriosus. Marked hearing loss required hearing aids. A few other deletions of the very terminal portion of 14q have been reported (Meschede et al. 1998). Although this is the only case in which hearing loss was documented, it is possible that hearing evaluations were not performed in all other cases.

2.2.3 Chromosomal Rearrangement Syndromes and Hearing Loss

The majority of chromosomal deletions result in partial monosomy for a particular chromosomal region. Deletions may result from the unbalanced segregation of a parental reciprocal translocation, or occur *de novo*.

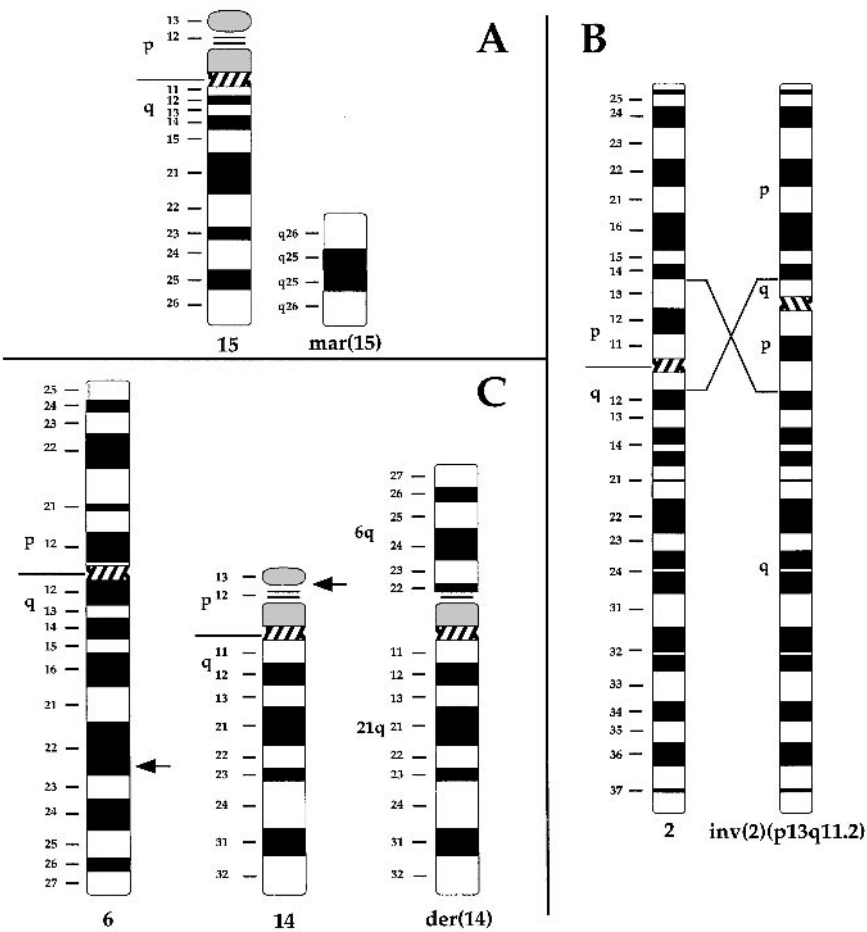


FIGURE 5.5. Novel chromosome rearrangements that cause hearing loss, among other physical findings. (A) Marker chromosome of an inverted duplication of 15q25–q26, on right, as compared with a normal chromosome 15 (left). The patient described (Huang et al. 1998) had two normal chromosomes 15 in addition to the marker 15. (B) Normal chromosome 2, on left, as compared with inverted chromosome 2 (Kozma et al. 1996). (C) Partial trisomy of 6q described by Conrad et al. (1998) as a result of a 6;14 translocation. Arrows indicate the chromosomal break-points. The child had two normal chromosomes 6 (on left), one normal chromosome 14 (middle) and one derivative 14, with additional 6q material attached to 14p.

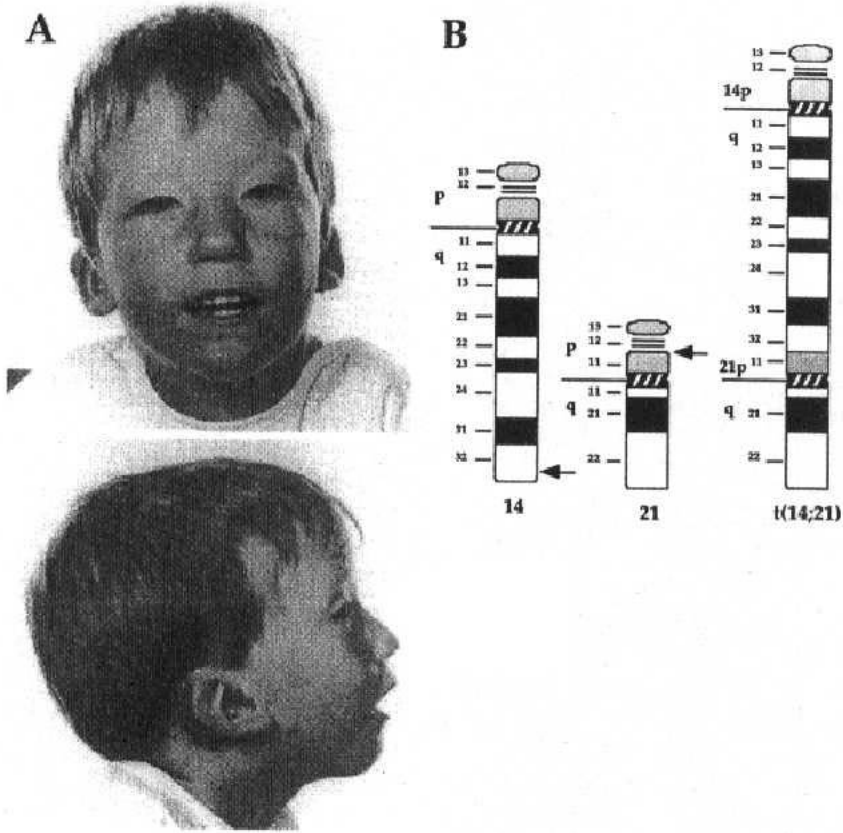


FIGURE 5.6. Translocation between chromosomes 14 and 21. (A) The patient at three years of age (From Meschede et al. 1998, Submicroscopic deletion in 14q32.3 through a *de novo* tandem translocation between 14q and 21p, American Journal of Medical Genetics 80:443–7, Copyright 1998 John Wiley & Sons, Inc. Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.). Notice the posteriorly rotated, low-set ears and the need for a hearing aide. (B) Ideograms representing the patient's translocation. Arrows indicate the chromosomal breakpoints. The child has one normal chromosome 14 (left), one normal 21 (middle), and one translocated chromosome, t(14;21) (right), which results in monosomy for the distal portion of 14q.

Terminal deletions, as well as many interstitial deletions, have been described (Therman and Susman 1993). Some deletions, such as those at 15q11-q13 or 22q11, associated with the Prader-Willi/Angelman syndromes or DiGeorge/velocardiofacial syndromes, respectively, are relatively common. Others, such as 5p- (cri-du-chat syndrome) or 4p- (Wolf-Hirschhorn syndrome), are rarely seen.

Many deletions or smaller microdeletions have been observed often enough to be categorized into known clinical syndromes, i.e., similar deletions producing similar phenotypic patterns. The following sections will

briefly discuss some of the deletion syndromes that have hearing loss as a frequent clinical finding.

2.2.3.1 1p36 Deletion Syndrome

1p36 deletion syndrome is a relatively newly recognized microdeletion syndrome (Fig. 5.7A). Because the deletion is usually fairly small, and in a large, lightly staining portion of the terminus of the short arm of chromosome 1,

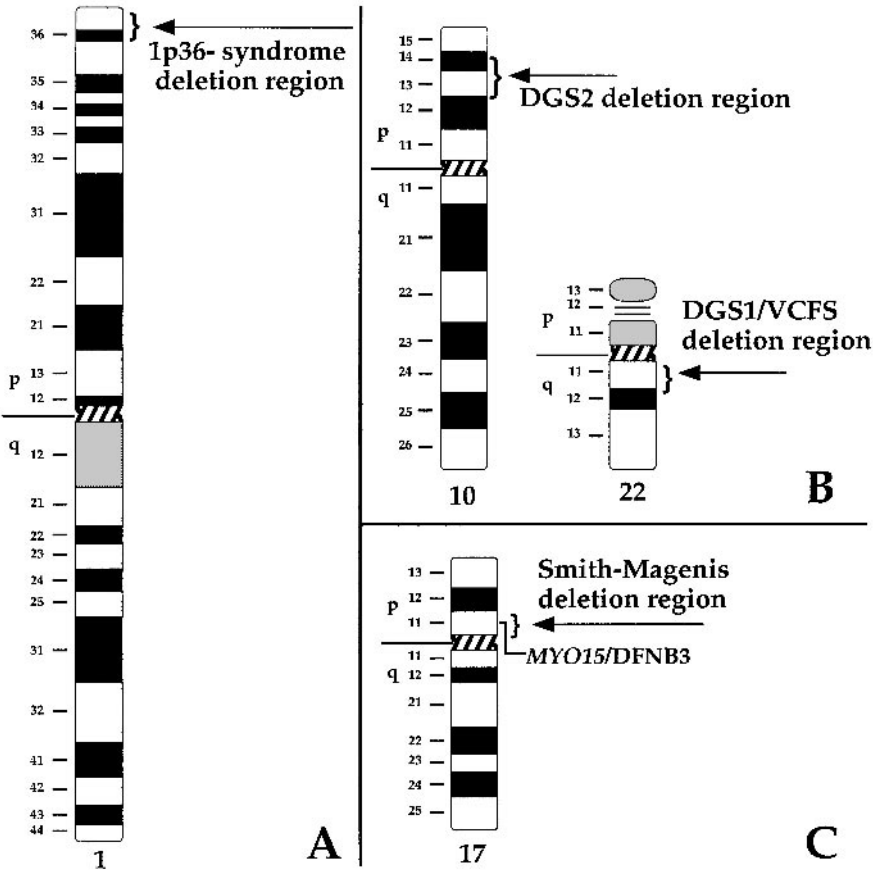


FIGURE 5.7. Microdeletion syndromes that have hearing loss as a component of the syndrome. (A) Ideogram of chromosome 1 showing the region of deletion on distal 1q that causes 1p36 deletion syndrome (Wu et al. 1999). (B) The regions on chromosomes 22 and 10 that are deleted in DiGeorge/velocardiofacial syndromes types *one* and *two*, respectively. (C) The region of the short arm of chromosome 17 that is deleted in Smith-Magenis syndrome. The nonsyndromic hearing disorder DFNB3 maps to this region; mutations in the gene for *MYO15* underlie this disorder (Wang et al. 1998).

the deletion may frequently be missed upon standard cytogenetic analysis. Shapira et al. (1997) estimate its incidence at more than 1 in 1,000, which would make 1p36 deletions one of the more common cytogenetic deletion syndromes. The physical findings in this syndrome can be somewhat vague and variable, depending on the size of the deletion. Hypotonia, moderate mental retardation, mild facial dysmorphism, abusive behavior, and hearing loss are all part of the spectrum of physical findings. Interestingly, a preponderance of maternally derived deletions has been observed (Wu et al. 1999). By studying a series of deletions of various sizes, Wu et al. (1999) describe the smallest deletion in which sensorineural hearing loss is present.

2.2.3.2 *DiGeorge Syndrome/Velocardiofacial Syndrome*

DiGeorge syndrome/velocardiofacial syndrome (DGS1/VCFS) was originally thought to be two separate entities until both were found to be caused by deletions in 22q11. Thus, they are now viewed collectively with variable phenotypes, depending on the deletion size and genetic background. Ninety percent of deletions occur *de novo* (Gardner and Sutherland 1996). Clinical findings include thymus deficiency, conotruncal heart anomalies, mildly dysmorphic facies, hypoparathyroidism, palatal anomalies and deafness (Hong 1998). Heart disease is the leading cause of death among DGS1/VCFS infants. The phenotype may be highly variable, and members of the same family, presumably with identical deletions, have variable expressivity of DGS1/VCFS features (Gardner and Sutherland 1996). The majority of patients are growth delayed, and most also experience learning disabilities (Gorlin et al. 1995). The underlying embryological defect is thought to be improper development of the facial neural crest tissues, resulting in defective neural pouch derivatives (Lammer and Opitz 1986).

Cytogenetic analysis of DGS1/VCFS individuals reveals a deletion on one chromosome 22 at band q11 in approximately 33% of cases (Fig. 5.7B). Often the deletion is cryptic, being so small that it can only be observed with molecular probes. By molecular studies, at least 90% of DGS1/VCFS patients have been found to have deletions.

The hearing loss seen in DGS1/VCFS can range from mild to severe and is usually conductive, though it can be sensorineural. Defects detected are often structural, owing to interference of development of the 3rd and 4th pharyngeal pouches (Ohtani and Schuknecht 1984). Mondini deformity is found, as well as malformed ossicles, and external auditory canal anomalies.

An additional DGS2/VCFS deletion locus is located on 10p. It has been found *de novo* as an interstitial or terminal deletion of 10p (Fig. 5.7B) (Schuffenhauer et al. 1998), or as the result of an inherited, unbalanced translocation (Hon et al. 1995). In addition to the spectrum of features found in DGS1/VCFS patients with the 22q11 deletion, patients with the 10p deletion can also demonstrate microcephaly, hand and foot anomalies, genitourinary defects, severe psychomotor retardation and sensorineural deafness (Shapira et al. 1994). Similar to individuals with 22q11 deletions,

10p patients can have highly variable expressivity of any or all of these findings. Schuffenhauer et al. analyzed the deletions of 12 DGS2 patients, three of whom had sensorineural hearing loss, and determined that the hearing loss gene(s) within the DGS2 locus on 10p must reside just distal to marker D10S1705 (Schuffenhauer et al. 1998).

2.2.3.3 *Smith-Magenis Syndrome*

Smith-Magenis syndrome is a contiguous gene-deletion syndrome originally described in 1982 and resulting from interstitial deletions in 17p11.2 (Fig. 5.7C). The phenotype includes brachycephaly, midface hypoplasia, prognathism, deep hoarse voice, psychomotor and growth retardation, and behavioral problems that include self-injurious behavior and sleep disorders (OMIM 1999). Greenberg et al. (1996) found that 68% of Smith-Magenis patients have hearing loss, with approximately two-thirds of those having conductive loss and one-third having sensorineural hearing loss.

The nonsyndromic autosomal-recessive hearing loss locus DFNB3 was mapped to the pericentromeric region of chromosome 17 (Friedman et al. 1995) and then refined to 17p11.2, within the deletion interval for Smith-Magenis syndrome (Liang et al. 1998). Later that same year, DFNB3 was shown to be caused by mutations in the gene encoding the unconventional myosin, *MYO15* (Wang et al. 1998). Interestingly, several Smith-Magenis patients who have sensorineural hearing loss and deletion of one allele of *MYO15* also have a point mutation in the remaining allele of *MYO15* (Liang et al. 1998). Deletion of the Smith-Magenis region uncovered the recessive mutation, making the patients hemizygous for the mutated allele.

2.3 *Use of Cytogenetics to Help Identify Candidate Genes*

An extremely useful aspect of cytogenetics when studying any genetic disease is that it can lead to identification or refinement of a disease locus. Chromosomal rearrangements or deletions that disrupt critical genes can be the first clue to the locus of a disease gene. This approach was successful in studies of Waardenburg syndrome type 1, where linkage analyses had been unproductive, apparently due to locus heterogeneity (Mueller, Van Camp, and Lench, Chapter 4). In both Branchio-Oto-Renal syndrome and X-linked mixed deafness, deletions found in the chromosomes of affected individuals allowed refinement of the disease intervals, ultimately leading to cloning of the genes.

2.3.1 *Waardenburg Syndrome Type 1*

Waardenburg syndrome is an autosomal-dominant disorder that commonly manifests as deafness with pigmentary anomalies. Ishikiriya et al. (1989) reported a Japanese child with a phenotype consistent with Waardenburg

syndrome type 1 (WS1), seen in Figure 5.8A. This child represented a new mutation in his family and, significantly, also had a *de novo* chromosome inversion involving the distal portion of the q arm of chromosome 2 including bands 2q35 and 2q37.3 (Fig. 5.8B). Prior to this report, WS1 had not been assigned to a chromosomal location. Earlier reports of possible linkage to the ABO blood group at 9q34 (Simpson et al. 1974) could not be confirmed. Using the genomic landmarks provided by $\text{inv}(2)(\text{q}35\text{q}37.3)$, Foy et al. (1990) established genetic linkage of WS1 to distal chromosome 2q, and

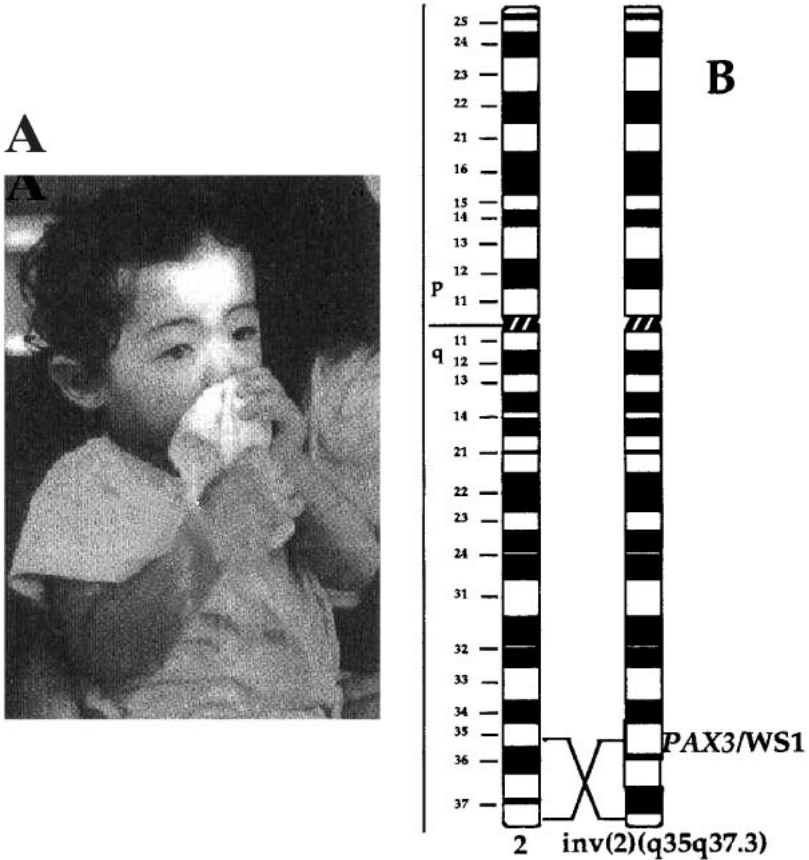


FIGURE 5.8. De novo chromosome rearrangement that was used to facilitate mapping the locus for Waardenburg syndrome type 1. (A) Japanese boy with WS1 in which a *de novo* chromosome 2 inversion was observed (From Ishikiriya et al. 1989, Waardenburg syndrome type I in a child with *de novo* inversion (2) (q35q37.3), American Journal of Medical Genetics 33:505–7, Copyright 1989 John Wiley & Sons, Inc. Reprinted by permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.). Notice the pigmentary anomalies of the skin and heterochromia irides. (B) Ideogram of chromosome 2, showing the inversion seen in this child and the position of the WS1 gene, *PAX3*, at the q35 inversion breakpoint.

suggested that WS1 may be homologous to the *Splootch* mouse that maps to a region of homologous synteny on mouse chromosome 1. The *Splootch* mouse has pigmentary anomalies similar to those seen in humans with WS1, but, interestingly, does not have any hearing deficit. When the mouse *Pax3* gene was cloned and shown to cause the *Splootch* phenotype (Epstein et al. 1991; Goulding et al. 1991), the human homolog, *HuP2* or *PAX3*, was evaluated as a candidate gene in several WS1 families by heteroduplex analysis (Mueller, Van Camp, and Lench, Chapter 4). Band shifts were observed that showed complete concordance with the WS1 phenotype. The *PAX3* gene was sequenced in these families and found to contain disease causing mutations (Tassabehji et al. 1992). To date, dozens of mutations in *PAX3* have been found that cause WS1. Identification of *PAX3* as the WS1 gene was facilitated greatly by the cytogenetic finding of the *inv(2)(q35q37.3)*.

2.3.2 X-Linked Mixed Deafness

Loci for a number of disorders have been mapped to Xq21 by linkage analysis and translocation studies, including retinal dystrophy choroideremia, mental retardation, cleft lip and palate, and mixed deafness with stapes fixation and perilymphatic gusher (DFN3) (OMIM 1999). DFN3 is the most common form of X-linked hearing impairment. Physical mapping of the Xq21 region using patients with these disorders and with cytogenetically visible deletions of Xq21 allowed successive refinement of the locus for DFN3 (Bach et al. 1992; Cremers et al. 1989), until ultimately it was reduced to 500 kb (Fig. 5.9) (Huber et al. 1994). When the murine *Pou3f4* gene was cloned and mapped to the mouse X chromosome in a region of homologous synteny with human Xq21 (Douville et al. 1994), *POU3F4* became a positional candidate gene for DFN3. Radiolabelled mouse *Pou3f4* probes hybridized to Southern blots of genomic DNA from DFN3 males with cytogenetically visible deletions, failed to detect any restriction fragments, suggesting the orthologous *POU3F4* gene was deleted in these individuals. The mouse sequence was used to make primers to amplify and clone the human *POU3F4* gene. SSCP analysis showed frameshift mutations in *POU3F4* in four DFN3 patients who did not have cytogenetically visible deletions (de Kok et al. 1995), confirming that loss of *POU3F4* in the deletion patients was responsible for their hearing loss. Additional DFN3 patients have been identified subsequently who have deletions in Xq21 that do not encompass *POU3F4*, but delete regions centromeric to the gene. These patients may harbor deletions in unidentified *POU3F4* regulatory sequences, or another gene whose product is similar to or interacts with the *POU3F4* protein (de Kok et al. 1996).

2.3.3 Branchio-Oto-Renal Syndrome

The autosomal dominant Branchio-Oto-Renal (BOR) syndrome is the association of branchial arch anomalies, such as branchial cysts or fistulas,

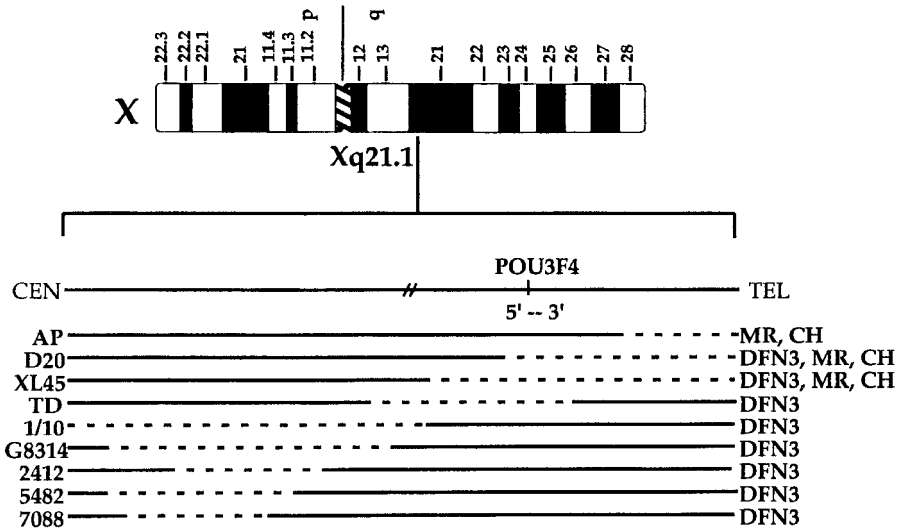


FIGURE 5.9. Deletion map of the X chromosome for band q21 (adapted from Cremers et al. 1989; Bach et al. 1992; Huber et al. 1994; de Kok et al. 1996). The position of the *POU3F4* gene, which is responsible for X-linked mixed deafness, is marked. Solid lines represent intact chromosomal fragments. Dashed lines represent deleted regions. Patient identifiers are listed on the left. Patient phenotypes are listed on the right. MR = mental retardation, CH = choroideremia, DFN3 = X-linked mixed deafness. Notice that deletions downstream of *POU3F4* also appear to cause DFN3 consistent with a positional effect on *POU3F4*.

renal anomalies including dysplasia or aplasia of one or both kidneys, and mild to profound hearing impairment that can be sensorineural, conductive or mixed (OMIM 1999). The gene for BOR syndrome was cloned in 1997 (Abdelhak et al. 1997), and is the human homolog of the *Drosophila* eyes absent gene. *EYAI* maps to 8q13, and its identification was greatly aided by the discovery in 1989 of a family with a chromosomal rearrangement of 8q. Members of this family suffered from both BOR syndrome and Tricho-Rhino-Phalangeal syndrome type 1 (TRPS1). Their 8q rearrangement involved a direct insertion of DNA from 8q13–q21 into 8q24 (dir ins(8)(q24.1q13.3q21.1)), shown in Figure 5.10 (Haan et al. 1989). TRPS1 had previously been associated with chromosomal breaks in 8q24 (Buhler and Malik 1984; Bowen et al. 1985), suggesting that the BOR phenotype resulted from gene disruption at either 8q13 or 8q21. Linkage analysis with other BOR families indicated that 8q13 was the more probable locus (Wang et al. 1994). Careful examination of 8q13 in the BOR/TRPS1 family revealed a small deletion of less than 1Mb at the break-point (Gu et al. 1996). Sequencing a contig spanning that break-point revealed the presence of a novel human gene homologous to the *Drosophila* eyes-absent gene. When the human *EYAI* gene was sequenced in other BOR patients,

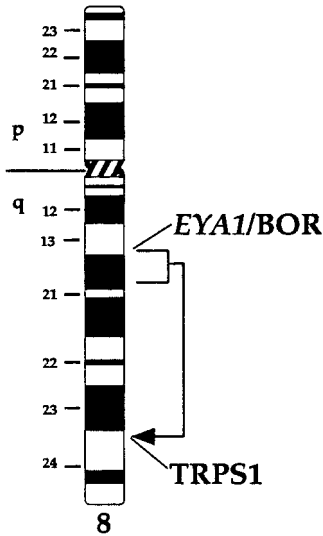


FIGURE 5.10. Ideogram of chromosome 8 showing regions of rearrangement in the family (described by Haan et al. 1989) that were instrumental in identifying the BOR (Branchio-Oto-Renal syndrome) locus. This family had both BOR syndrome and Tricho-Rhino-Phalangeal syndrome type 1 (TRPS1). A portion of 8q from q13.3 to q21.1 was inserted in band q24.1, apparently causing disruption of both the BOR gene (*EYA1*) and the TRPS1 locus.

eight different mutations were found, including frameshifts and splice-site variants, confirming that deletion of *EYA1* in the family with the *dir* ins(8)(q24.1;q13.3q21.1) was pathogenetic for the BOR phenotype (Abdelhak et al. 1997).

3. Expressed Sequence Tags

Another technique for identifying disease genes is the analysis of expressed sequence tags (ESTs). The human genome is estimated to contain 50,000 to 100,000 genes, only a fraction of which have been identified. Over 90% of human disease genes identified by positional cloning experiments have exact sequence matches to one or more ESTs in GenBank, demonstrating that sequence tags are useful identifiers of medically important genes. The current collection of human ESTs has been estimated to represent over 60,000 distinct human genes; thus, much progress has been made in the effort to identify all human genes.

ESTs are short stretches of cDNA sequence, usually less than 500 base pairs (Adams et al. 1991). Because the sequence is derived from cDNA, the gene represented was transcribed in the tissue from which the original mRNA was isolated. ESTs provide a sequence handle to identify and clone the complete gene, and give insight into which genes are expressed in various tissues or cell types. Adams et al. (1991) originally described 600 ESTs from a human brain cDNA library; today there are over 4,500,000 ESTs in GenBank (<http://www.ncbi.nlm.nih.gov/dbEST/>), derived from more than 100 different cDNA libraries.

As well as providing sequence tags, ESTs can be used to map human genes, without knowledge of the gene's function. A sequence tagged site (STS) is created from an EST by mapping the EST using radiation hybrid panels. Radiation hybrid panels are collections of somatic cell hybrid clones, usually hamster-human hybrids, in which irradiated human genomic DNA was fused with hamster cells. Each hamster cell line incorporated different small fragments of human DNA, so that only human DNA markers that are physically very close to one another are likely to be present in the same hamster line. Thus, detection of an EST in a cell line indicates that it is close to the markers that are known to be present in that cell line.

3.1 Cochlear cDNA Libraries and Cochlear Expressed Sequence Tags

Cochlear ESTs have several uses. They provide sequence tags that enable identification, mapping and cloning of cochlear-expressed genes, both those that are unique to the cochlea, and those expressed in many or all tissues. This may prove to be a particularly valuable tool because a number of deafness loci have been mapped by linkage analysis (Mueller, Van Camp, and Lench, Chapter 4), but the genes have yet to be cloned.

Certainly, human cochlear tissue is a notoriously difficult tissue from which to obtain good quality mRNA for the generation of cDNA libraries. The first human cochlear cDNA library was created by Robertson et al. (1994). This library, known as the Morton Fetal Cochlear cDNA Library, has resulted in the generation of over 4,000 human cochlear ESTs from 3,200 individual clones (Skvorak et al. 1999). All the cochlear EST sequences can be found in dbEST, and all the clones are commercially available.

In addition to the Morton human cochlear cDNA library, a number of rodent and avian cochlear cDNA libraries have also been developed. Guinea pig or chick cochleae, while small and difficult to isolate, are still far easier to obtain than human cochleae. Additionally, because cochlear tissues can be obtained from animals at various time points, developmental processes can be studied in animal models that cannot be attempted using human tissues.

3.1.1 Known Genes Expressed in the Human Cochlea

The sequences of all of the ESTs generated from the Morton fetal cochlear cDNA library were compared with sequence data in the various GenBank databases. This analysis revealed that 33% were very similar to previously identified human genes (Skvorak et al. 1999). In all, significant similarity to 517 known human genes was found. Because the library had not been subtracted or normalized in any way, many of the known genes were "housekeeping" genes. A complete list can be found at <http://hearing.bwh.harvard.edu>. Several of the cochlear ESTs were from

genes known to cause either syndromic or nonsyndromic hearing loss. For example, the genes for BOR (*EYA1*), DFNB3 (*GJB2*), and DFNA9 (*COCH*) are among the cochlear ESTs. Interestingly, genes for other hearing disorders, for example human *diaphanous* (DFNA1) and *POU4F3* (DFNA15), which are known to be expressed in fetal cochlea, were not among the cochlear ESTs; this is because the library was not exhaustively sequenced, and less abundant messages were thus less likely to be detected. Sequencing of additional cochlear clones would provide a more complete survey of all the genes expressed in this tissue, and such an effort is currently underway.

3.1.2 Cochlear-Specific ESTs

Sequence matches were not detected in GenBank for 540 cochlear ESTs. These clones may represent genes that are uniquely expressed in the inner ear. It is perhaps not surprising that an organ as complex as the cochlea would express exclusively a large number of genes. Further investigations using tissue hybridization studies and cell-type-specific cDNA libraries are required to determine whether these genes are widely expressed in cochlea, or confined to a specific cell type.

3.1.3 Map Locations of Cochlear ESTs Provide Candidate Genes for Hearing Disorders

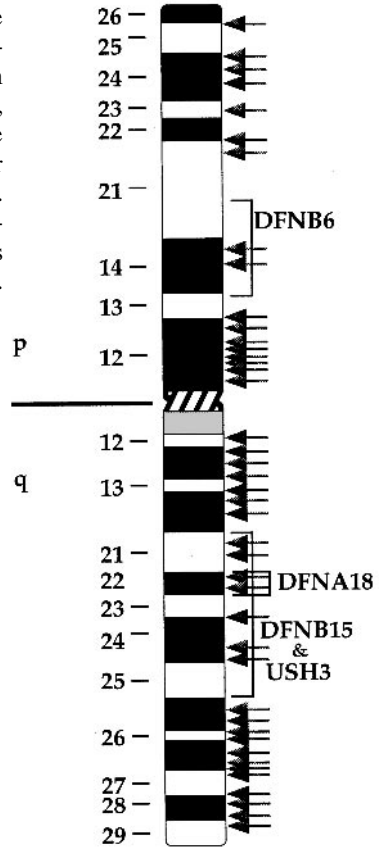
Many genes for nonsyndromic deafness are yet to be identified. Because of small pedigree sizes, geographically isolated populations and the difficulty in grouping kindreds because of genetic heterogeneity, a precise location of the gene cannot be determined by linkage analysis (Mueller, Van Camp, and Lench, Chapter 4). The task of identifying the pathogenetic sequence in a chromosomal segment can be quite daunting. Clearly, positional-candidate genes and ESTs within the region of interest are the first to be investigated, with a high priority being given to those that are known to be expressed in the cochlea.

Several hundred of the cochlear ESTs have been mapped. Of these, 57 map to the genetic intervals of 22 different syndromic and nonsyndromic hearing disorders (Skvorak et al. 1999), making them immediate positional candidates for these disorders (Fig. 5.11). This approach successfully identified *COCH* as the defective gene in DFNA9 deafness (Robertson et al. 1998).

3.2 Other Cochlear Libraries

Several cochlear libraries have been constructed from various rodent or avian cochlear tissues. Creating libraries from model organisms has two advantages. First, because much research in the molecular biology of hearing is conducted in model organisms such as rodents and birds, cDNA

FIGURE 5.11. Ideogram of human chromosome 3, showing the loci of four human hearing disorders for which the responsible genes remain to be identified: *DFNB6*, *DFNA18*, *DFNB15*, and Usher syndrome type 3. Arrows indicate the approximate positions of human cochlear expressed sequence tags (ESTs) (Skvorak et al. 1999) that map to chromosome 3. Map locations for these and additional cochlear ESTs can be found at <http://hearing.bwh.harvard.edu>.



libraries from these animals' inner ears are directly useful for investigating genes involved in hearing and hearing loss in these animals. In several cases, the human orthologs of genes first identified in mouse have been shown to cause hearing loss in humans. Second, although rodent and bird cochleae are small and difficult to dissect, there is a plentiful supply and the tissue can be dissected immediately after the animal is sacrificed. This is in stark contrast to human cochlear tissue, which is extremely difficult to obtain and is often partially degraded due to postmortem autolysis.

The rodent and chick cochlear cDNA libraries that have been constructed to date have mostly been made in an effort to isolate genes that are uniquely expressed in the inner ear. The first reported was a guinea pig organ of Corti cDNA library (Wilcox and Fex 1992). Since then, at least four rat inner ear libraries have been described, including one from mRNA extracted exclusively from outer hair cells (Harter et al. 1999). The remainder were made from whole cochleae (Ryan et al. 1993; Beisel and Kennedy 1994; Soto-Prior et al. 1997). In each case, several hundred cochleae were used as starting material. Two chicken cochlear cDNA libraries have been

developed recently (Oberholtzer et al. 1994; Heller et al. 1998), as well as a mouse cochlear cDNA library constructed from 400 mouse cochleae (Crozet et al. 1997).

4. Summary

Thus far, cytogenetic analysis has been used in only a small number of studies of patients with hearing loss. This reflects the fact that chromosomal studies are costly, and karyotypic anomalies are likely to account for a low percentage of cases. In addition, only a relatively small proportion of molecular biologists have any expertise with cytogenetic techniques. Appropriately, cytogenetics is not the first technique to be considered when evaluating a child with nonsyndromic deafness. However, cytogenetic testing could be valuable in cases of deafness of unknown etiology, particularly if there were accompanying congenital anomalies, or a family history of multiple spontaneous abortions. When all other causes of deafness, either genetic or acquired, are eliminated, cytogenetics could be used to determine if the hearing loss may be due to a chromosome rearrangement, such as a balanced translocation. The advantage would be that, if such a chromosome rearrangement were found, it would immediately suggest the location of the deafness gene.

The cochlear ESTs and libraries that exist have been extremely helpful in understanding different aspects of the molecular biology of the inner ear. Many of the deafness genes that have been cloned thus far have originated from one of these libraries, or been shown to be expressed in the inner ear by virtue of being found in one of the cochlear libraries. The current cochlear EST collection will be augmented in the near future by the production of additional cochlear ESTs, providing an enriched biological resource for investigating gene expression in the inner ear.

Acknowledgments. The writing of this manuscript was supported by NIH grants R01 DC-03402 to C. C. M. and DC-01076 to M. C. King.

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6

Autosomal and X-Linked Auditory Disorders

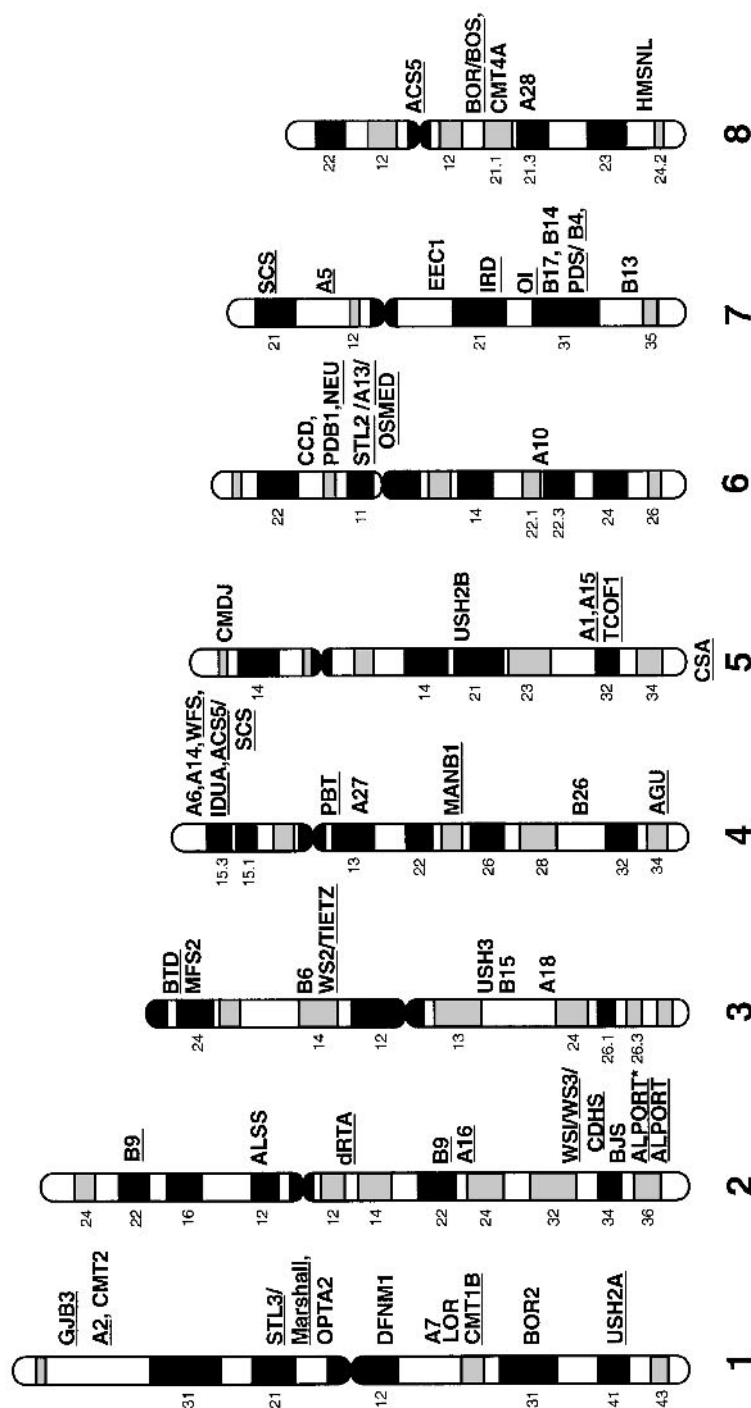
ANDREW J. GRIFFITH and THOMAS B. FRIEDMAN

1. Introduction

Mutations in any one of more than two hundred different genes can cause hearing loss (Fig. 6.1). This remarkable genetic heterogeneity is a reflection of the great diversity of highly specialized proteins and cell types required for the electromechanical transduction of sound stimuli within the auditory system (Rubsamen and Lippe 1998). This chapter describes our rapidly evolving knowledge of the genes responsible for syndromic and nonsyndromic deafness. Two treatises of enduring merit predate the recent identification of many of the genes for syndromic forms of deafness, but their detailed clinical and classical genetic descriptions remain of great value (Gorlin et al. 1995; Konigsmark and Gorlin 1976).

The incidence of significant hearing loss among newborns is approximately 1/1,000 and about 50% of these cases appear to have a genetic etiology (Liu et al. 1994; Marazita et al. 1993; Morton 1991). The proportion of persons with late-onset hearing loss with a genetic origin is not known. Many families have been described in which parents transmit defective genes to their offspring, resulting in hearing loss at birth or later in life (Fraser 1976; Konigsmark 1969; Nance and Sweeney 1975; Reardon et al. 1992). Dominant and recessive modes of inheritance were noted. The majority of nonsyndromic sensorineural hearing loss is recessive, 20% is dominant, and 1 to 3% is X-linked (Marazita et al. 1993). In each of a large number of multiplex families, the pattern of inheritance of hearing loss implicates a single major Mendelian trait, either sex-linked, autosomal dominant or autosomal recessive. Non-Mendelian inheritance of deafness due to mitochondrial mutations has also been reported and is the subject of Chapter 7.

Hearing loss can be caused by environmental insults such as perinatal trauma, prolonged loud noise exposure and barotrauma, injuries to the skull, radiation, as well as intrauterine or postnatal exposure to ototoxic drugs or infectious agents (Garetz and Schacht 1996; Nadol 1993; Prasher 1998). Susceptibility to some of these insults has an underlying genetic



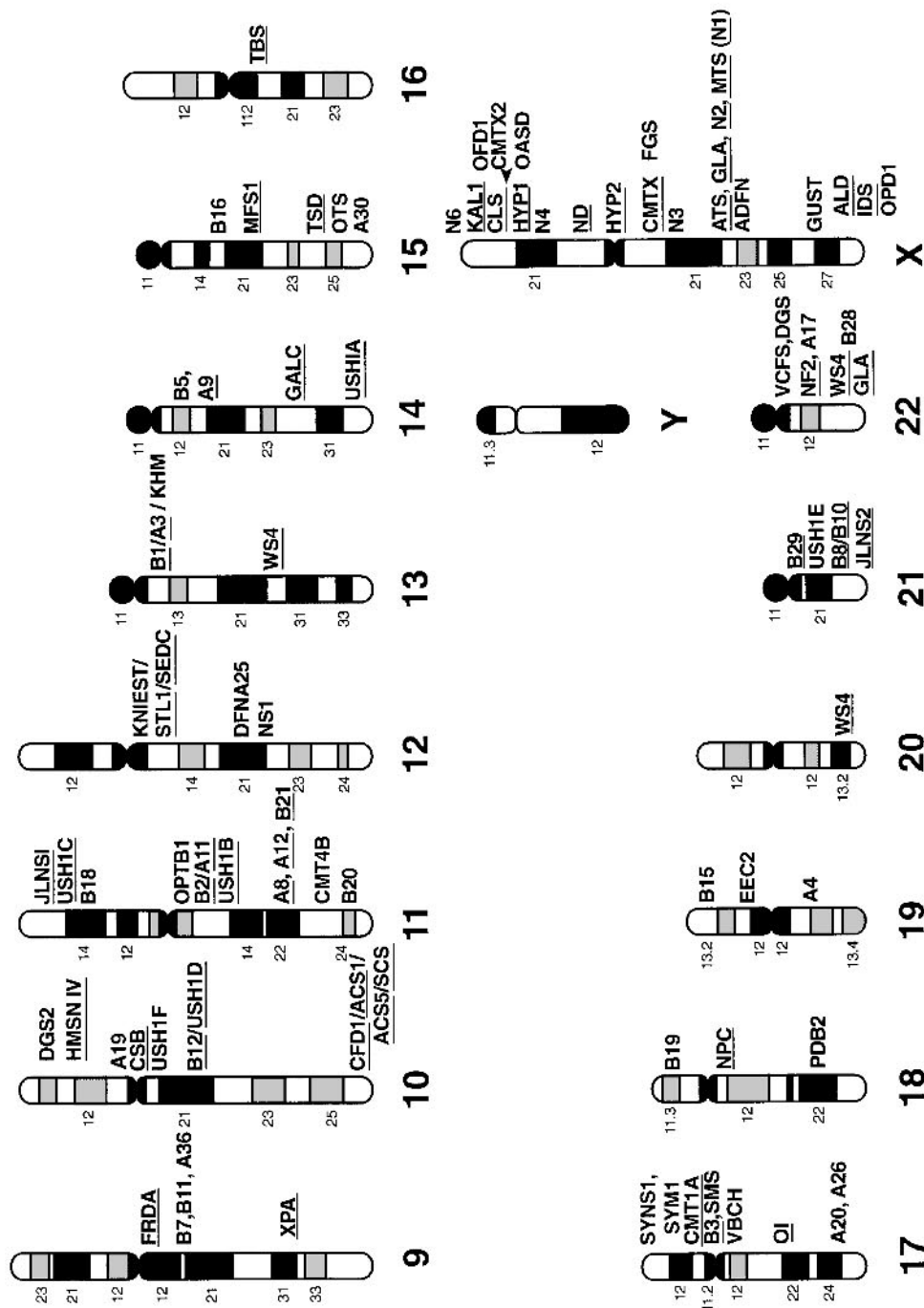


FIGURE 6.1. Diagram of a normal male karyotype (46,XY) showing the location of the centromeres, the chromosome arms (short or p-arm and long or q-arm) and the major Giemsa-bands. The autosomes are numbered from 1 to 22. The designations for DFNA loci are shown as A1 to A36, while DFNB loci are shown as B1 to B28. The symbols for syndromic and nonsyndromic hearing loss loci that have been identified are underlined. When the same gene has been identified with mutant alleles associated with more than one syndrome and/or more than one nonsyndromic locus, the loci are separated by a *slash (/)*. Loci separated by a comma map to a similar chromosomal interval. Notice that loci for syndromic and nonsyndromic deafness are found on all the autosomes and X-chromosome. The gene symbols for the syndromic deafness loci are listed in Table 6.5, along with a brief description of the phenotype. ACS1, Apert syndrome; ACS5, Pfeiffer syndrome; ADFN, Albinism-deafness syndrome; AGU, Aspartylglucosaminuria; ALD, Adrenoleukodystrophy; ALSS, Alström syndrome; BJS, Björnstad syndrome; ATS, X-linked Alport syndrome; BOR, Branchio-oto-renal syndrome; BOR2, Branchio-otic syndrome with commissural lip pits; BOS, Branchio-otic syndrome; BTD, Biotinidase deficiency; CCD, Cleidocranial dysplasia; CDHS, Craniofacial-deafness-hand syndrome; CFD1, Crouzon syndrome; CLS, Coffin-Lowry syndrome; CMDJ, Craniometaphyseal dysplasia, Jackson type; CMT1A, Charcot-Marie-Tooth disease, type 1A; CMT1B, Charcot-Marie-Tooth disease, type 1B; CMT2, Charcot-Marie-Tooth disease, type 2; CMT4A, Charcot-Marie-Tooth disease, type 4A; CMT4B, Charcot-Marie-Tooth disease, type 4B; CMTX, Charcot-Marie-Tooth disease, X-linked dominant; CMTX2, Charcot-Marie-Tooth disease, X-linked recessive; CSA, Cockayne syndrome, type I/A; CSB, Cockayne syndrome, type II/B; dRTA, Renal tubular acidosis with sensorineural deafness; DGS, DiGeorge syndrome; DGS2, DiGeorge syndrome; EEC1, Ectrodactyly, ectodermal dysplasia, and cleft lip/palate syndrome, type I; EEC2, Ectrodactyly, ectodermal dysplasia, and cleft lip/palate syndrome, type II; FGS, FG syndrome; FRDA, Friedreich ataxia type I; GALC, Krabbe disease; GJB3, gap junction protein β -3; GLA, Fabry disease; GUST, Gustavson syndrome; HMSN IV, Refsum disease; HMSNL, Hereditary motor and sensory neuropathy, Lom type; HYP1, Hypophosphatemia Type I; HYP2, Hypophosphatemia Type II; IDS, Hunter syndrome; IDUA, Hurler syndrome; IRD, Refsum disease, infantile form; JLNS1, Jervell and Lange-Nielsen syndrome; JLNS2, Jervell and Lange-Nielsen syndrome; KHM, Vohwinkel syndrome, classic form; KAL1, Kallmann syndrome; LOR, Vohwinkel syndrome, variant form; MANB1, Beta mannosidosis; Marshall, Marshall syndrome MFS1, Marfan syndrome; MFS2, Marfan syndrome; MTS, Mohr-Tranebjaerg syndrome, Jensen syndrome; ND, Norrie disease; NEU, Sialidosis; NF2, Neurofibromatosis type 2; NPC, Niemann-Pick type C disease; NS1, Noonan syndrome; OASD, Ocular albinism with sensorineural deafness; OFD1, Orofaciodigital syndrome, type 1; OI, Osteogenesis imperfecta; OPD1, Otopalatodigital syndrome, type I; OPTA2, Osteopetrosis type II; OPTB1, Osteopetrosis; OSMED, Chondrodystrophy with sensorineural deafness; OTS, otosclerosis; PBT, Piebaldism; PDB1, Paget disease; PDB2, Paget disease; PDS, Pendred syndrome; SCS, Saethre-Chotzen syndrome; SEDC, Spondyloepiphyseal dysplasia congenita; SMS, Smith-Magenis syndrome; STL1, Stickler syndrome; STL2, Stickler syndrome; STL3, Stickler syndrome; SYM1, Symphalangism; SYNS1, Multiple synostoses syndrome; TBS, Townes-Brooks syndrome; TCOF1, Treacher Collins' syndrome; TIETZ, Tietze's syndrome; TSD, Tay-Sachs disease; USH1A, Usher syndrome type 1A; USH1B, Usher syndrome type 1B; USH1C, Usher syndrome type 1C; USH1D,

Usher syndrome type 1D; USH1E, Usher syndrome type 1E; USH1F, Usher syndrome type 1F; USH2A, Usher syndrome type 2A; USH2B, Usher syndrome type 2B; USH3, Usher syndrome type 3; VBCH, van Buchem disease; VCFS, Velocardiofacial syndrome; WFS, Wolfram syndrome; WS1, Waardenburg syndrome type 1; WS2, Waardenburg syndrome type 2; WS3, Waardenburg syndrome type 3; WS4, Waardenburg syndrome type 4; XPA, Xeroderma pigmentosum.

component (Fischel-Ghodsian 1998). Since hearing loss can be acquired, it is to be expected that, among hearing impaired members of a large family with a high incidence of deafness, some individuals may have hearing loss due to a nongenetic cause. These individuals are said to be phenocopies (Hadorn 1961). Audiological evaluations may not be able to distinguish between hereditary and acquired deafness, in which case the information derived from a thorough clinical history is invaluable.

The ongoing identification and inevitable elucidation of the functions of the genes for hearing loss provides entry points toward an integrated understanding of auditory system structure, function, and development. From a public health perspective, the insights gained from molecular genetic studies may guide the development of strategies to slow the rate of progressive hearing loss. Even without specific medical treatment, knowledge of impending hearing loss allows timely speech and communication rehabilitation. It is also anticipated that results of genetic tests will be used to counsel individuals who are at risk for hearing loss.

2. Classification and Evaluation of Hearing Loss

2.1 Audiometric

Pure-tone audiometry can be used to classify hearing loss as conductive, sensorineural, or mixed, which is a combination of conductive and sensorineural. Conductive hearing loss is associated with pathology affecting any of the anatomic components that mechanically transduce sound to the cochlea. Thus, abnormalities of the external ear, ear canal, tympanic membrane (eardrum), ossicles, oval window (the interface of the ossicular chain with the cochlea), round window, or middle ear space may cause conductive hearing loss. Sensorineural hearing loss may be associated with dysfunction of any of the components of the auditory pathway that convert the physical stimulus of sound into an electrical stimulus that is transmitted to the auditory cortex. Sensorineural hearing loss may therefore be caused by lesions of the cochlea, auditory (cochlear; eighth cranial) nerve, auditory brainstem, or even higher order auditory structures within the brain.

The severity of hearing loss is also routinely categorized. Measured pure-tone hearing thresholds may be used to classify hearing loss as mild (26 to

40 dBHL), moderate (41 to 55 dBHL), moderately severe (56 to 70 dBHL), severe (71 to 90 dBHL), or profound (greater than 90 dBHL). Thresholds less than 25 dBHL are considered normal. The degree of hearing loss is often not uniform across the tested frequencies (typically 250 Hz to 8 kHz). For example, there may be normal hearing at low and middle frequencies, with severe hearing loss at high frequencies. This type of hearing loss is described as high-frequency or down-sloping sensorineural hearing loss, since the line connecting the pure-tone threshold levels slopes down in the higher frequencies, which are recorded on the right side of an audiogram. Sensorineural hearing loss may also be low-frequency or up-sloping (Lesperance et al. 1995), or it may be U-shaped, predominantly affecting middle frequencies. A flat audiogram refers to relatively similar thresholds across the tested frequencies.

Figure 6.2 illustrates several of the many possible distinct audiometric patterns of hearing loss. These descriptions provide concise and useful categorizations of audiometric findings, but in practice many audiograms do not fit neatly into any one category. Furthermore, the degree and type of hearing loss between two ears of a given patient may be asymmetric. In a family segregating a gene causing hearing loss, subjective evaluations of audiometric data are sometimes required to distinguish between an affected and an unaffected individual. This distinction needs to be made before genotyping begins, since mistakes in assigning affection status can jeopardize a genetic analysis, particularly in families with progressive or late-onset deafness. Based on data of hearing thresholds of age- and sex-matched normal-hearing individuals, Govaerts and coworkers (1998) have proposed an algorithm to more objectively differentiate between affected and unaffected persons. Furthermore, age- and sex-specific reference ranges for hearing levels and longitudinal changes are available for a Caucasian population of 681 men and 416 women (Morrell et al. 1996). The subjects were rigorously screened for otological disorders and noise exposure in order to remove these confounding variables and provide a normative data set.

2.2 *Clinical*

It is important to distinguish between syndromic and nonsyndromic hearing impairment. Specifically, it should be noted whether the hearing loss is inherited in association with any other disorders or abnormalities, even if they do not fit neatly into any previously described syndromes. An additional distinction regards the onset of hearing loss, which is often described in relation to the development of speech because adequate auditory function is important for the development of spoken language. Hearing impairment occurring prior to speech development is called prelingual, whereas that occurring after speech development is called postlingual. Given the wide range of age of onsets of postlingual hearing loss, this term is impre-

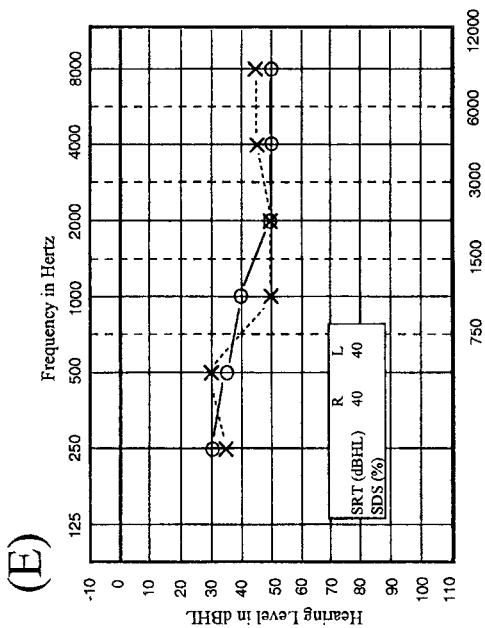
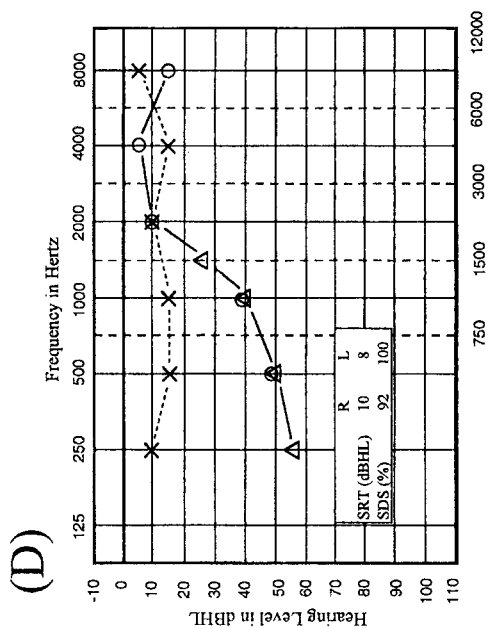
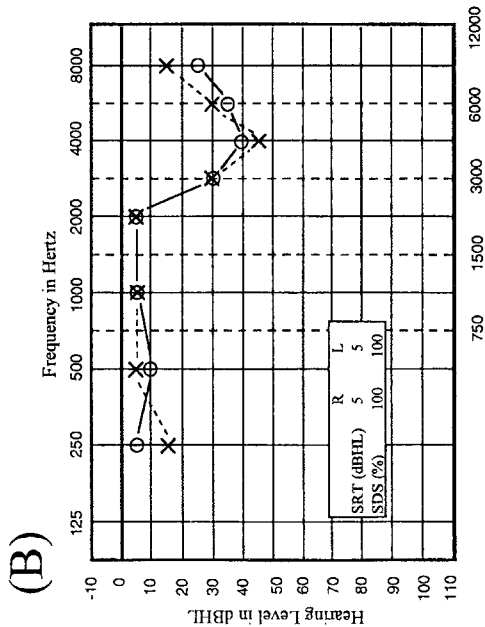
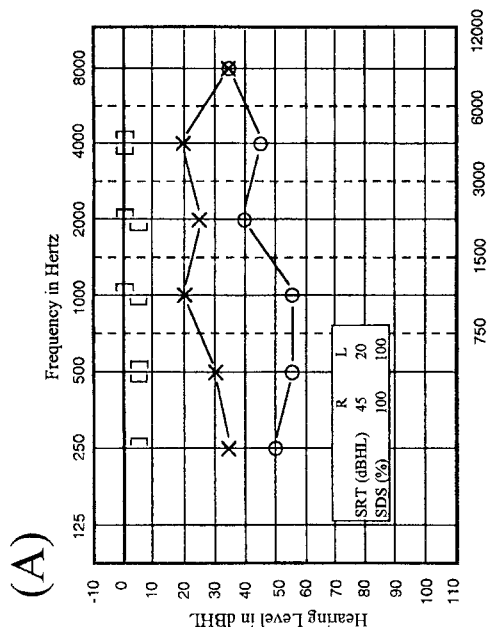
cise and, for most purposes, probably best replaced by a simple description of age of onset (e.g., onset in adolescence, third decade, etc.).

Except in cases of congenital profound deafness, the temporal course of the hearing impairment is also described. Hearing loss may be stable or progressive, and progression is often observed in association with dominant inheritance of hearing loss (Table 6.1). Fluctuation may be present in other types of hearing loss, such as that associated with recurrent otitis media or Meniere's disease (see *DFNA9*, 3.7.2). Fluctuating or incremental hearing loss may also be associated with head trauma or congenital inner ear malformations such as Mondini dysplasia or enlarged vestibular aqueducts, or a combination of these two factors (Jackler and De La Cruz 1989; Levenson et al. 1989; Schuknecht 1980). These inner ear malformations sometimes have a genetic basis, as there have been several reports of familial cases (Abe et al. 1997; Chan et al. 1991; Griffith et al. 1996; Griffith et al. 1998).

2.3 Temporal Bone Histopathology

Inner ear neurosensory tissue for histologic examination is almost never accessible in the living patient. Therefore, histopathologic studies of post-mortem human temporal bone specimens can be helpful for correlating anatomic and histologic findings with clinical observations in the myriad disorders affecting auditory and vestibular function (Schuknecht 1993). However, it has been difficult to derive broad conclusions about the pathogenesis of hereditary hearing loss due to the paucity or absence of specimens for many of the disorders, as well as artifactual changes arising from delayed or inadequate preservation of the specimens.

FIGURE 6.2. Pure-tone audiograms: (A) Bilateral conductive hearing loss associated with bilateral otitis media; (B) Bilateral high-frequency sensorineural hearing loss due to noise exposure, demonstrating a typical "noise notch" at 4,000 Hz; (C) Unilateral moderate to profound sensorineural hearing loss in a patient who received intratympanic gentamicin (aminoglycoside therapy); (D, E, F) Differing patterns of sensorineural hearing loss in three affected members of a single kindred segregating Waardenburg syndrome. Panel D illustrates a unilateral low-frequency hearing loss, panel E illustrates a symmetric, fairly flat mild to moderate loss, and panel F shows a profound right-sided loss with a left-sided, mild to moderate high-frequency loss. SRT is the speech reception threshold, the softest level at which a person can understand 50% of spoken words. SDS is the speech discrimination score, the percentage of word stimuli that are perceived correctly. Although bone conduction thresholds are not shown in B and E, previous audiometric evaluations on these patients had demonstrated that the hearing loss was sensorineural, with no significant difference between bone- and air-conduction thresholds. Reliable speech discrimination scores were not obtained as part of the evaluations shown in C and E due to the young age of the patients.



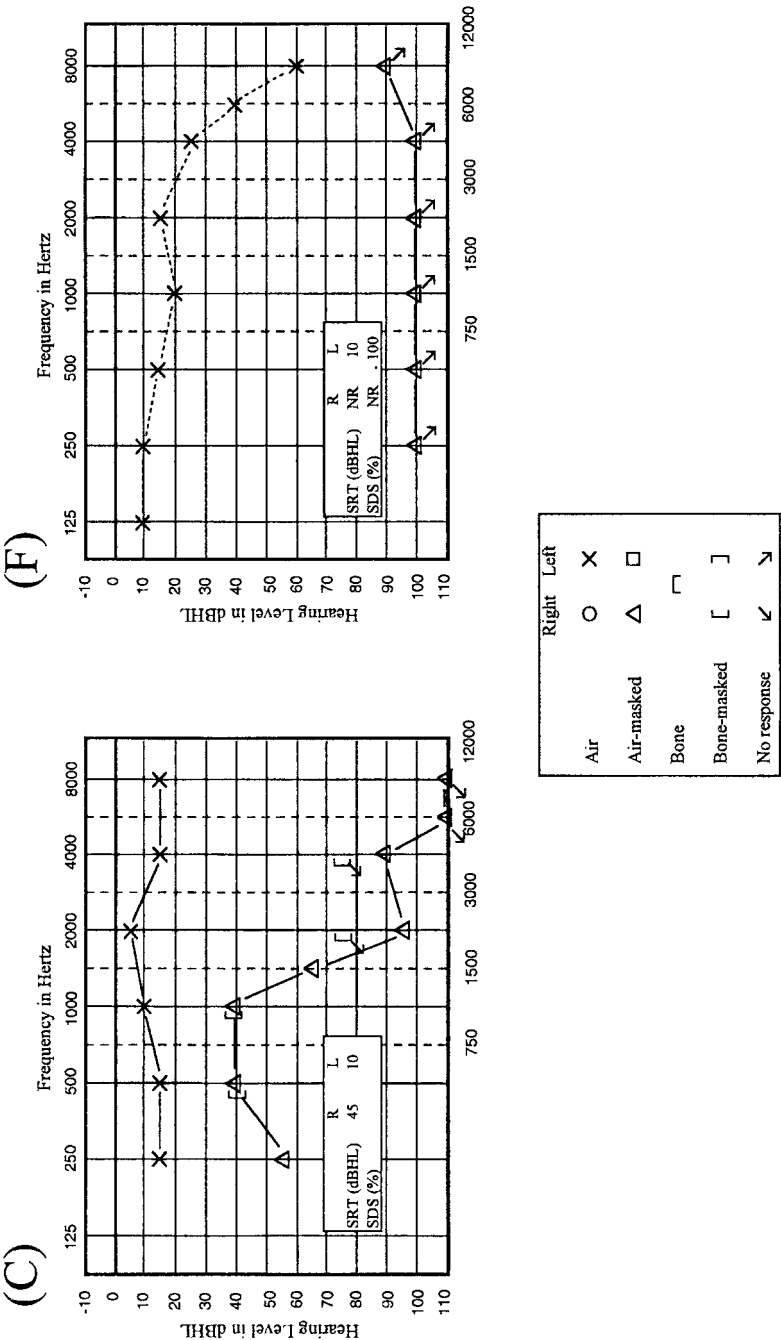


TABLE 6.1. Nonsyndromic autosomal dominant deafness loci (DFNA)

Locus	Location	Gene (see Table 6.4)	Onset of Hearing Loss (yr)	Phenotype/Comments	Mouse Model	Selected References
<i>DFNA1</i>	5q31	<i>HDIA1</i>	~10	Progressive SNHL to profound levels by age 40yrs; initially affects low frequencies	<i>sy</i>	Leon et al. 1981 Leon et al. 1992
<i>DFNA2</i> ^a	1p34-p35	<i>GJB3</i> (<i>Cx31</i>)	Variable, 10-30	Progressive SNHL; 30 to >55 dBHL by age 30 to 40yrs; initially affects high frequencies		Coucke et al. 1994 Van Camp et al. 1997 Coucke et al. 1999 Liu et al. 2000 Xia et al. 1998
<i>DFNA3</i> ^a	1p34	<i>et c.</i> <i>KCNQ4</i>	Variable, congenital; 10	Progressive SNHL of 1 dB/yr, initially affects high frequencies		Coucke et al. 1999
	13q12	<i>GJB2</i> (<i>Cx26</i>) (see DFNB1)	Approximately 4	All frequencies affected, stable or slowly progressive SNHL; 50% profound, 50% with 70 to 100 dBHL	<i>Gjb2</i> knockout	Kubisch et al. 1999 Chaib et al. 1994 Denoyelle et al. 1997
	13q12	<i>GJB6</i> (<i>Cx30</i>)	20-40	Mild to moderate progressive SNHL, initially affects high frequencies in a small family		Gabriel et al. 1998 Grifa et al. 1999
	19q13		20-30	Fluctuating, progressive SNHL to profound levels by fourth decade; all frequencies affected	<i>quivering, qv</i>	Chen et al. 1995
<i>DFNA5</i>	7p15	<i>DFNA5</i>	20-30 5-15	Progressive SNHL; initially affects high frequencies		Van Laer et al. 1997 Van Laer et al. 1998
<i>DFNA6</i>	4p16.3		5-15	Progressive SNHL to 40 to 50 dBHL by 15yrs; initially affects low frequencies	<i>tilted, tlt</i>	Lesperance et al. 1995
<i>DFNA7</i>	1q21-q23		6-15	Progressive SNHL to >45 dBHL by 15yrs; initially affects high frequencies		Fagerheim et al. 1996
<i>DFNA8/12</i>	11q22-24	<i>TECTA</i> (see DFNB21)	Prenatal, early childhood	Stable mild-severe SNHL		Verhoeven et al. 1997 Balciuniene et al. 1998 Verhoeven et al. 1998

<i>DFNA9</i>	14q12-q13	<i>COCH</i>	6-62	Progressive SNHL to profound levels by age 50yrs; mucopolysaccharide deposition in neural channels of inner ear	Manolis et al. 1996 Robertson et al. 1998 de Kok et al. 1999
<i>DFNA10</i>	6q22-q23		20-50	Progressive SNHL to moderate to severe levels	O'Neill et al. 1996
<i>DFNA11</i>	11p12.3-q21	<i>MYO7A</i>	~10	Gradually progressive SNHL to moderate levels by age 20 to 60yrs	Tamagawa et al. 1996 Liu et al. 1997c Liu et al. 1998
<i>DFNA13</i>	6q21		20-40	Progressive SNHL	Brown et al. 1997
<i>DFNA14</i>	4p16		10-20	Progressive SNHL affecting low and mid frequencies	Van Camp et al. 1999
<i>DFNA15</i>	5q31-q33	<i>POU4F3</i>	~15-20	Progressive SNHL to moderate to severe levels by 50 to 80yrs	Vahava et al. 1998
<i>DFNA16</i>	2q23-q24.3		6-	Fluctuating, progressive SNHL; responds to prednisone therapy	Fukushima et al. 1998
<i>DFNA17</i>	22q12.2-q13.3		~10	Progressive SNHL, initially affects high frequencies; cochleosaccular degeneration	Lalwani et al. 1997 Lalwani et al. 1999
<i>DFNA18</i>	3q24		Congenital	Progressive SNHL to mild to moderate levels	Boensch et al. 1998
<i>DFNA20</i>	17q25		11-30	Progressive SNHL; initially affects high frequencies, then mid-high frequencies; low frequencies near normal at age 40yr	Morell et al. 2000
<i>DFNA23</i>	14q21-q22		Prelingual	MHL in 50% of affected members; stable in the majority of affecteds; moderate to profound loss in high frequencies	Salam et al. 1999
<i>DFNA24</i> <i>OTS</i> otosclerosis	4q35-qter 15q25-q26		Prelingual ~30	Stable SNHL in mid-high frequencies Progressive CHL; MHL in 10% of affected individuals	Hafner et al. 2000 Tomek et al. 1998
				Cartilage matrix deficiency, <i>cmd</i>	

^a DFNA2 and DFNA3 are examples of locus heterogeneity (i.e., more than one deafness gene in a genetic map interval); underlined mouse mutant strain is known to be the orthologue of the human locus, otherwise mouse model is speculative. SNHL, Sensorineural hearing loss; CHL, conductive hearing loss; MHL, mixed hearing loss; dBHL, decibels hearing loss. DFNA loci published in peer-review journals are included in this table.

2.4 *Mouse Models of Human Deafness*

It is much easier to perform controlled studies of hereditary auditory disorders in mice, whose inner ear is sufficiently similar to that of humans to serve as a model system for such disorders. Mice are much easier to study in large numbers and at controlled time points during fetal and postnatal life, and postmortem artifacts are minimized by expeditious fixation of the tissues. The histopathologic findings associated with mouse deafness mutations were reviewed (Steel et al. 1987; Steel and Bock 1983) and a classification scheme was presented for inner ear abnormalities similar to that proposed by Ormerod for cochlear abnormalities in human temporal bones (Ormerod 1960).

Steel and Bock proposed three major patterns of inner ear pathology, including morphogenetic abnormalities, which are gross structural deformities of the inner ear. One example of this type of malformation that occurs in humans is the Mondini inner ear deformity (Schuknecht 1980). The second group comprises neuroepithelial abnormalities in which the primary defect appears to occur in the organ of Corti. In these mutants, the stria vascularis is normal, the endocochlear potential is present, Reissner's membrane is in normal position, the auditory abnormalities are fairly symmetric between the ears, and the vestibular neurosensory end organs are variably affected (Fig. 6.3). The third pattern of abnormalities is cochleosaccular dysplasia, in which the primary defect appears to be in the stria vascularis, and the endocochlear potential is thought to be absent (Fig. 6.3). The organ of Corti may degenerate secondarily, Reissner's membrane eventually collapses, the saccular membrane may also collapse, and the ears are often asymmetrically affected. This pattern also occurs in human temporal bones, where it is known as Scheibe dysplasia (Scheibe 1892). Although the scheme described by Steel and Bock is based upon generalized observations of mice, they provide a cognitive framework for interpreting similar studies of human hereditary hearing loss (Steel and Bock 1983).

2.5 *Many Families and Multiple Alleles*

In a family with one affected individual (sporadic), or in a family with just a few affected individuals, distinguishing between an acquired or a genetic etiology is difficult, and attempting to assign a mode of inheritance is problematic. In a large multigeneration family, or in an isolated population with many affected individuals, it is possible to determine the mode of inheritance by segregation analysis with greater certainty. Segregation analysis is a statistical evaluation of the mode of inheritance based on the transmission ratios of a trait in a family (Thompson 1986) (Tables 6.1, 6.2, 6.3). If segregation analysis suggests a simple Mendelian trait and the family is large, identifying a map position for the disease gene is now a routine enterprise that is becoming highly automated.

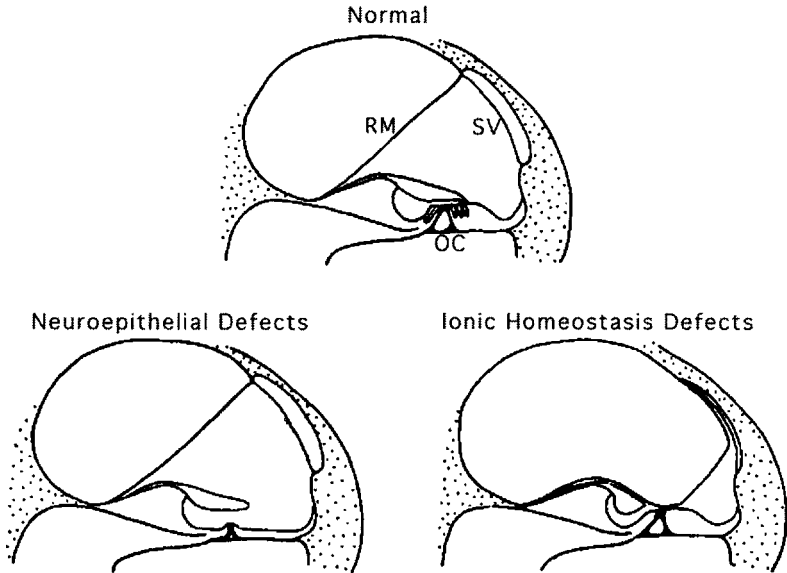


FIGURE 6.3. Cochlear histopathologic patterns associated with hereditary hearing impairment in mice (adapted from Steel and Bock 1983). Neuroepithelial defects are characterized by a Reissner's membrane (RM) that is in normal position, and an initially normal-appearing stria vascularis (SV). Ionic homeostatic defects (also known as cochleosaccular degeneration) may include a primary strial abnormality, which leads to collapse of Reissner's membrane and secondary damage to the organ of Corti (OC).

The value of having many unrelated families segregating mutations at the same nonsyndromic or syndromic deafness locus cannot be overstated. Additional families linked to the same locus are a potential source of informative meiotic recombinants that may narrow the chromosomal interval encompassing the disease gene. Among unrelated linked families, multiple alleles are suggested when there is more than one haplotype in the region of the disease gene. Multiple independently arising mutant alleles also provide a stronger argument for a causal connection between the mutated gene and the hearing-loss phenotype.

3. DFNA, DFNB and DFN Loci

3.1 General Remarks

When "DFN", the acronym for deafness, is followed directly by a number (e.g., *DFN2*), the locus maps to the X-chromosome. When "DFN" is followed either by "A" or "B," the mutant allele is an autosomal dominant

TABLE 6.2. Nonsyndromic autosomal recessive deafness loci (DFNB)

Locus	Location	Gene (see Table 6.4)	Onset of Hearing Loss	Phenotype/Comments	Mouse Model	Selected References
<i>DFNB1</i>	13q12	<i>GJB2</i> (Cx26)	Congenital to late onset	Progressive SNHL, mild to profound	<i>Gjb2</i> knockout	Guilford et al. 1994a Brown et al. 1996
<i>DFNB2</i>	11q13.5	<i>MYO7A</i>	Congenital to early onset	SNHL, mild to profound	<i>shakerZ, sh-1</i>	Weil et al. 1997
<i>DFNB3</i>	17p11.2	<i>MYO15</i>	Congenital	SNHL, profound	<i>shakerZ, sh-2</i>	Friedman et al. 1995 Liang et al. 1998 Li et al. 1998
<i>DFNB4</i>	7q31	<i>PDS</i>	Congenital	Profound SNHL; some patients with enlarged vestibular aqueducts		
<i>DFNB5</i>	14q12		Congenital	Severe to profound SNHL		Fukushima et al. 1995a
<i>DFNB6</i>	3p14-p21		Congenital	Profound SNHL	<i>spinner, sr</i>	Fukushima et al. 1995b
<i>DFNB7</i>	9q13-q21		Congenital	Profound SNHL	<i>deafness, dn</i>	Jain et al. 1995
<i>DFNB8</i>	21q22		Postlingual	Profound SNHL by age 16 yrs		Veske et al. 1996
<i>DFNB9</i>	2p22-p23	<i>OTOF</i>	Congenital	Profound SNHL before second year of life		Chaib et al. 1996a Leal et al. 1998
<i>DFNB10</i>	21q22.3		Congenital	Severe to profound SHNL		Bonné-Tamir et al. 1996
<i>DFNB11</i>	9q13-q21		Congenital	profound SNHL		Scott et al. 1996; Greinwald et al. 1997
<i>DFNB12</i>	10q21-q22		Prelingual	Profound SNHL		Chaib et al. 1996b
<i>DFNB13</i>	7q34-36		Prelingual	Severe SNHL; probably progressive	<i>waltzer, v</i>	Mustapha et al. 1998a
<i>DFNB14</i>	7q31		Prelingual	Profound SNHL		Mustapha et al. 1998b
<i>DFNB15</i>	3q21.3-q25.2		Prelingual	Profound SNHL	<i>mocha, mh</i>	Chen et al. 1997
<i>DFNB16</i>	19p13.3-p13 15q15-q21		~5-7 yrs	Mild to severe SNHL		Campbell et al. 1997
<i>DFNB17</i>	7q31		Congenital	Profound SNHL		Villamar et al. 1999
<i>DFNB18</i>	11p15.1-p14		Congenital	Profound SNHL		Greinwald et al. 1998
<i>DFNB20</i>	11q25-qter		Congenital—1 yr	Moderate to profound SNHL; joint contractures		Jain et al. 1998 Moynihan et al. 1999
<i>DFNB21</i>	11q	<i>TECTA</i>	Prelingual	Severe to profound SNHL		Mustapha et al. 1999
<i>DFNB26</i>	4q		Congenital	Profound SNHL		Riazuddin et al. 1999

Underlined mouse mutant strain is known to be the orthologue of the human locus, otherwise mouse model is speculative. SNHL, sensorineural hearing loss. DFNB loci published in peer-review journals are included in this table.

TABLE 6.3. X-linked nonsyndromic deafness loci (DFN)

Locus	Location	Onset of Hearing Loss in Males	Additional Comments about Phenotype	Selected References
<i>DFN1/MTS^a</i>	Xq22	Early onset to 1.5 yrs	Progressive SNHL in males; 80 dBHL by age 13 yrs. accompanied by variation in spasticity, dystonia, ataxia, mental retardation, hip fractures and progressive visual disability leading to blindness; mild or no hearing loss in females	Tranebjaerg et al. 1995 Jin et al. 1996 Koehler et al. 1999
<i>DFN2</i>	Xq22	Congenital	Profound SNHL in males; mild to moderate hearing loss in female carriers	Tyson et al. 1996
<i>DFN3</i>	Xq13-q21.1	Infancy	Progressive MHL in males, with or without stapes fixation; perilymphatic gusher upon stapes surgery	de Kok et al. 1995 de Kok et al. 1996 Minowa et al. 1999
<i>DFN4</i>	Xp21.2	Congenital	Profound SNHL in males; variable expression in carrier females	Lalwani et al. 1994 Pfister et al. 1998 Pfister et al. 1999 del Castillo et al. 1996
<i>DFN6</i>	Xp22	Postlingual to 5–7 yrs	Progressive SNHL in males mainly affecting high frequencies evolving to severe to profound loss at all frequencies in adulthood; incomplete penetrance and variable expression in female carriers	

^aSee Mohr-Tranebjaerg syndrome in Table 6.5, since DFN1 was shown to be syndromic. Abbreviations: SNHL, sensorineural hearing loss; MHL, mixed hearing loss; dBHL, decibels hearing loss

or recessive locus, respectively. DFNA and DFNB loci have a numeric suffix, a consecutively increasing Arabic number indicating the order in which the DFNA, DFNB, or DFN locus was mapped (<http://www.gene.ucl.ac.uk/nomenclature/>). This nomenclature reveals nothing about the chromosomal address of the gene or the associated audiological phenotype, except in the case of X-linked DFN loci that map to the X chromosome. After publication of a new DFN locus, the gene is given a unique six-digit MIM number (see <http://www3.ncbi.nlm.nih.gov/Omin/> or the printed version (McKusick 1998)).

Although recessive mutations of deafness genes usually cause congenital profound deafness (Tables 6.1 and 6.2), dominant alleles, with a few exceptions, are associated with progressive hearing loss. Mutations of dosage-sensitive genes would be expected to be dominant via a mechanism known as haploinsufficiency. Alternatively, the product of a dominant mutant allele might “poison” the function of the wild-type gene product via a dominant negative mechanism. For example, the function of a multimeric complex normally comprised of wild-type gene products may be disrupted by the incorporation of the product of a mutant allele of that gene.

Twenty-eight DFNB loci have been mapped, and six have now been identified: *DFNB1*, *DFNB2*, *DFNB3*, *DFNB4*, *DFNB9* and *DFNB21*. Thirty-five DFNA loci have been mapped and ten have been identified that map to nine loci: *DFNA1*, *DFNA2*, *DFNA3*, *DFNA5*, *DFNA8/12*, *DFNA9*, *DFNA11*, *DFNA13* and *DFNA15* (Table 6.4). The first part of this review is primarily focused on those DFNA and DFNB loci that have been identified.

Surprisingly, many of the DFNA and DFNB genes are expressed in tissues other than the auditory system (Table 6.4), yet mutant alleles seem only to cause nonsyndromic hearing loss. In some families thought preliminarily to be segregating nonsyndromic hearing loss, there may well be other clinical features that were missed initially and become obvious when more is known about the tissue and developmental expression profiles of the causative gene. Alternatively, a mutant phenotype may be restricted to the auditory system because other tissues in which the gene is expressed have functional redundancy for that particular gene product. Other gene products with similar functions presumably compensate for disruption of function of the mutated gene.

The rigor of proof of identification varies for the deafness genes that have been identified to date. To demonstrate that a disease gene has been identified, a candidate gene must reside within the critical map interval of the disease gene and, if fully penetrant, the mutant allele co-segregates with the disease. Mutant alleles that alter protein sequence or expression pattern in a biologically significant manner are not usually common polymorphisms, and are absent in a random sampling of several hundred individuals. However, a high carrier rate does not necessarily mean the variant is a benign polymorphism. Some mutant alleles of disease genes have a high

TABLE 6.4. Molecular genetics and expression patterns of identified DFNA, DFNB and DFN loci

Locus	Gene Symbol	Name of Protein	Expression Pattern	Proposed Function and Protein Domains	Affected Auditory Structure	Selected References
<i>DFNA1</i>	<i>DIAPH1</i>	diaphanous 1	Cochlea, brain, heart, placenta, lung, kidney, pancreas, liver, skeletal muscle	DIAPH1, member of the formin gene family involved in cytokinesis, cell polarity and actin polymerization	Hair cell cytoskeleton	Lynch et al. 1997
<i>DFNA2</i>	<i>KCNQ4</i>		Outer hair cells of the cochlea and the heart	Potassium channel		Kubisch et al. 1999
	<i>GJB3</i>	connexin 31	Skin, inner ear	Gap junction		Xia et al. 1998 Liu et al. 2000
<i>DFNA3</i> and <i>DFNB1</i>	<i>GJB2</i> (Cx26)	connexin 26	Cochlea; liver, pancreas, many other tissues	Gap junction	Support cell, spiral limbus, spiral ligament stria vascularis	Kelsell et al. 1997 Kumar and Gilula 1996 Kikuchi et al. 1995 Lautermann et al. 1998 Van Laer et al. 1998
<i>DFNA5</i>	<i>DFNA5</i>	<i>DFNA5</i>	Cochlea, lymphoblastoid cells; brain, heart, placenta, kidney			
<i>DFNA9</i>	<i>COCH</i>	Coch	Cochlea and vestibular tissue	vonWillebrand type-A- like domain; serine protease domain		Robertson et al. 1998
<i>DFNA8</i> and <i>DFNA12</i>	<i>TECTA</i>	α -tectorin	Inner ear	Structural protein	Tectorial membrane	Verhoeven et al. 1998
<i>DFNA11</i> , <i>DFNB2</i> , and <i>USH1B</i>	<i>MYO7A</i> (<i>shaker-1</i>)	myosin VIIA	Inner ear and retina	Intracellular movement; myosin motor IQ, MyIb4, talin-like domains, kinesin tail homology	Hair cell	Hasson et al. 1995 Liu et al. 1998 Weil et al. 1997

TABLE 6.4. *Continued*

Locus	Gene Symbol	Name of Protein	Expression Pattern	Proposed Function and Protein Domains	Affected Auditory Structure	Selected References
<i>DFNA15</i>	<i>POU4F3</i>		Cochlea	Transcription factor; target genes not identified	Bmn3c mouse KO is deaf and circles; lack hair and support cells; show inner ear neuronal degeneration	Vahava et al. 1998
<i>DFNB3</i>	<i>MYO15</i> (<i>shaker-2</i>)	myosin XV	Inner ear and pituitary; low levels in kidney, brain, testes	Intracellular movement; long N-terminal domain and myosin motor, IQ, Myth4, SH3, FIRM domains	Based on sh-2 mouse, inner and outer hair cells have short stereocilia	Wang et al. 1998 Probst et al. 1998 Liang et al. 1999
<i>DFNB4</i> and Pendred	<i>PDS</i>	pendrin	Thyroid and inner ear (endolymphatic duct and sac, areas of the utricle and sacculus, external sulcus within the cochlea)	Chloride/iodide anion transporter	Mondini malformation, enlargement of the vestibular aqueducts,	Everett et al. 1997 Li et al. 1998 Scott et al. 1999 Everett et al. 1999
<i>DFNB9</i>	<i>OTOF</i>	otoferlin	Inner ear, brain, placenta, liver, pancreas, skeletal muscle and kidney	Hypothesized to be involved in synaptic vesicle-plasma membrane fusion	Hair cells	Yasunaga et al. 1999 Yasunaga et al. in press
<i>DFN3</i>	<i>BRN-4</i>	POU3F4	Fibrocytes of the inner ear	POU-domain transcription factor	Based on Brn-4 KO, alterations in cochlear fibrocytes	deKok et al. 1995

carrier frequency. For example, among Caucasians, the carrier frequency is 2 to 3% for the $\Delta F508$ mutant allele of *CFTR*, the gene for cystic fibrosis (Morral et al. 1994), and among Ashkenazi Jews there is a carrier frequency of 4% for the 167delT mutant allele of *GJB2* (Morell et al. 1998).

Other types of observations strengthen the argument that a disease gene has indeed been identified, although these issues sometimes cannot be addressed easily. For example, animal models with a mutation in the orthologue of the human gene that approximates the human disease phenotype can be instrumental in understanding the pathophysiology. Moreover, correction of the mutant phenotype in an animal model by introducing the wild-type gene is a definitive demonstration that the disease gene has been correctly identified (Probst et al. 1998).

Finally, there are now cautionary tales suggesting that assignment of the “nonsyndromic” classification may be misapplied in the absence of thorough clinical data (Baldwin et al. 1995; Tranebjaerg et al. 1995) and it is certainly premature if the most common syndromes are not ruled out. The “nonsyndromic” designation for a mutant allele causing deafness should be considered tentative until the gene is identified and its spatial expression pattern is known. For nonsyndromic deafness genes that are expressed in tissues other than the auditory system, there may be associated subclinical features that were initially unnoticed.

3.2 *DFNA1*

3.2.1 Genetics

In an extended Costa Rican family, autosomal-dominant, progressive sensorineural hearing loss was inherited from two affected brothers who migrated to Costa Rica from Spain in the eighteenth century. Low-frequency hearing begins to be lost in the first decade, with profound losses at all frequencies occurring by age 30 (Lalwani et al. 1998; Leon et al. 1981). The causative gene, *DFNA1*, was found to be linked to markers defining a ~ 7 cM critical interval on 5q31 (Leon et al. 1992). This was the first autosomal nonsyndromic hearing loss gene to be mapped.

3.2.2 *Diaphanous* is *DFNA1*

DFNA1 was positionally cloned and identified as the human homologue of the *Drosophila diaphanous* gene (Lynch et al. 1997). Human *diaphanous*, *HDIA1*, is composed of 26 exons encoding a 140kDa protein (E. Lynch, personal communication). A G-to-T substitution in the consensus splice donor site of the penultimate exon was found in all 78 affected members of the Costa Rican family, but not in 330 normal-hearing members of this family. The G-to-T substitution results in aberrant splicing of exon 25 at a cryptic site 4 nucleotides into the intron. Splicing at this cryptic site causes a frameshift with substitution of 21 aberrant amino acids for the 32 wild-

type *HDIA1* amino acids at the carboxy terminus of the protein (Lynch et al. 1997).

The function of *HDIA1* (*Diaphanous*) in the auditory system, as well as the pathophysiology of the *HDIA1* mutation, are not known. *HDIA1* is a member of the formin family of genes that are important for normal cytokinesis and establishment of cell polarity. Functional clues about *HDIA1* may come from the fruit fly, *Drosophila melanogaster*, where a null allele of *diaphanous* is a pupal-lethal, a hypomorphic allele causes male sterility, and a weak allele results in polyploid cells in the central nervous system. It has been suggested that human *diaphanous* interacts with profilin to regulate actin filament formation, thereby affecting the intricate actin cytoskeleton of hair cells (Lynch et al. 1997). Any model for the function of human *diaphanous* in the inner ear should incorporate the delayed onset and progressive nature of the hearing loss associated with the *HDIA1* mutation observed in the Costa Rican kindred.

3.3 *DFNA2*

The *DFNA2* locus was mapped to 1p34 in several large families from Indonesia, the United States, Belgium and the Netherlands that were segregating dominantly inherited, progressive hearing loss (Coucke et al. 1994; Van Camp et al. 1997b). This chromosomal interval contains two good deafness candidate genes, one encoding a potassium channel (*KCNQ4*) and the other a gap junction family member, *GJB3* (connexin 31, *Cx31*). Subsequently, affected individuals in two small Chinese families with hearing loss were reported to have dominant mutations in *GJB3*, R180X and E183K, a nonsense mutation and a missense mutation, respectively. These postulated dominant mutations do not co-segregate perfectly with the hearing loss in two small Chinese pedigrees (Xia et al. 1998). For example, an unexplained nonpenetrant individual segregating the R180X mutation indicates that the R180X is not the pathogenic mutation and/or that there are other factors modifying the hearing-loss phenotype. Therefore, the genetic mechanism of dominant *GJB3* mutations such as R180X cannot be explained by a simple dominant mutation at this single locus.

Unfortunately, it is not possible to perform linkage analysis studies to corroborate these conclusions. The two Chinese pedigrees with a total of four affected individuals are too small to achieve a statistically significant Lod score. Nevertheless, recessive mutant alleles of *GJB3* do appear to be associated with hearing loss. Compound heterozygous mutant alleles of *GJB3* (R180X and E183K) were reported to co-segregate with probable recessive hearing loss in two small Chinese pedigrees (Liu et al. 2000). However, the recessive deafness mutations of *GJB3* in this study also lack corroborating linkage analysis.

DFNA2 is an example of locus heterogeneity in that mutant alleles of two closely linked genes, *GJB3* and *KCNQ4*, appear to be associated with

hearing loss. *KCNQ4* encodes a potassium channel expressed in the outer hair cells of the cochlea. Mutations of *KCNQ4* do co-segregate with dominant, progressive deafness in Dutch, Belgian and United States families. Linkage analyses of these families define the *DFNA2* interval (Coucke et al. 1999). In a small pedigree with dominant, progressive, nonsyndromic deafness, a missense mutation (G258S) was reported that exerts a dominant negative effect on the wild-type *KCNQ4* allele and affects endolymph potassium secretion (Kubisch et al. 1999).

Phenocopies were noted in two of the *DFNA2* families (Van Camp et al. 1997b). These individuals were omitted from genetic analyses to map the responsible genes. It is critical to identify phenocopies early in a mapping study, since even one unrecognized phenocopy in a large family may confound a positional cloning strategy to identify the gene.

3.4 *DFNA3*

GJB6 (connexin 30, *Cx30*) is another member of the large β -class family of gap-junction proteins that mediate intercellular communication. *GJB6* is closely linked to *GJB2* on chromosome 13q12, and many *GJB2* mutations causing nonsyndromic hearing loss have been identified (see *DFNB1*). Connexin 30 is expressed in the cochlea (Lautermann et al. 1998) and is structurally similar to connexin 26. Moreover, there are families with deafness linked to 13q12, but without mutations in *GJB2*. Thus, screening for mutation in *GJB6* is a logical experiment. However, Kelley and co-workers (Kelley et al. 1999) did not find mutations in *GJB6* in 23 dominant and 88 recessive nonsyndromic hearing-loss families, although these families are presumably too small for linkage analyses.

In a small Italian family with three individuals showing a pattern of dominant inheritance of progressive high frequency hearing loss, a missense mutation (T5M) was reported in the three affected individuals (Grifa et al. 1999). A convincing argument is yet to be made because this family is small. The T5M variant did not induce electrochemical coupling between cells when expressed in *Xenopus* oocytes. This assay, however, may not always be a reliable indicator of in vivo function. For example, the M34T allele of *GJB2* also has a low level of coupling in the *Xenopus* oocyte assay, yet there is an individual who is compound-heterozygous for M34T, and a null allele (167delT) who has essentially normal hearing (Griffith et al. 2000a).

3.5 *Genetics and Identification of DFNA5*

Van den Wijngaart described a Dutch family in which 105 members had highly variable, progressive high-frequency hearing loss, with an age of onset between 5 and 15 years and an autosomal-dominant pattern of inheritance (van den Wijngaart et al. 1985). The gene was initially mapped to a

15 cM region, which was further refined to 2 cM on 7p15 and, subsequently, the critical interval narrowed to 600 to 850 kb (Van Laer et al. 1997). Of the three expressed sequence tags (ESTs) identified in the *DFNA5* candidate region, one of these genes was found to have 10 exons and was predicted to encode a 499 amino acid protein containing no domains or motifs in common with proteins of known function. A Southern blot of genomic DNA from normal and affected individuals from the *DFNA5* family was prepared and probed with the EST clone derived from this gene. This blot and subsequent PCR analyses revealed a 1,189 base pair (bp) deletion in intron 7 of all affected individuals, but not in normal-hearing individuals of this family.

Why would a deletion of intronic sequence cause a mutant phenotype? One possibility is that the deletion affected normal mRNA splicing. This hypothesis was tested by reverse transcription–polymerase chain reaction (RT-PCR) analysis of mRNA from lymphoblastoid cell lines derived from affected and unaffected individuals. PCR amplification of the cDNA products with a forward primer in exon 7 and a reverse primer in exon 9 generated a 314-bp fragment in unaffected controls and a 121-bp fragment from affected individuals. Sequence analysis indicated that exon 8 was skipped in *DFNA5* mRNA from affected individuals. The function of *DFNA5* in the auditory system and in other tissues where it is expressed is unknown (Table 6.4).

3.6 *DFNA8, DFNA12 and DFNB21 are the Same Locus*

DFNA8 and *DFNA12* were mapped to an overlapping region on chromosome 11q22–q24 in one Austrian and one Belgian family, respectively, each segregating dominant nonsyndromic hearing loss (Fig. 6.1). CT scans of the temporal bones revealed no gross abnormalities of the inner ear. Hearing loss in affected members of the Belgian kindred was prelingual with stable moderate to severe losses in all frequencies. This is an unusual phenotype for dominant nonsyndromic hearing loss loci, since most mutant alleles of *DFNA* loci cause postlingual progressive hearing loss (Table 6.1).

DFNA12 mapped to a 36 cM interval on chromosome 11q (Verhoeven et al. 1997). A chromosomal region of this size may encompass hundreds, if not thousands, of genes. Identification of the responsible gene is a daunting endeavor unless there is a compelling candidate gene. *TECTA* is the homologue of mouse *Tecta* on chromosome 9. Based on conserved synteny, *TECTA* was predicted to be on human chromosome 11q22–24 in the interval to which *DFNA12* mapped. *TECTA* encodes α -tectorin, a noncollagenous extracellular protein found exclusively in the inner ear, located primarily in the tectorial membrane where it is cross-linked to β -tectorin. The genomic DNA sequence of the 23 exons of *TECTA* was screened by SSCP analysis for mutations in affected individuals in the two families used

to map *DFNA8* and *DFNA12* (Verhoeven et al. 1998). Two missense mutations, L1820F and G1824D, in exon 17 were found in the Belgian family and a missense mutation, Y1870C, was found in exon 18 in the Austrian family. Eighteen affected members of the Belgian family had the two adjacent missense mutations, and eight affected members in the Austrian family had the Y1870C mutation. These mutations were absent in the unaffected family members and in 100 representative normal controls, thus ruling them out as common polymorphisms.

Dominant mutant alleles of *TECTA* may account for the hearing-loss phenotype either through a dominant negative mechanism in which defective α -tectorin disrupts the structure of the tectorial membrane, and/or haploinsufficiency of *TECTA* may occur as a result of destabilization of mutant *TECTA* mRNA (Verhoeven et al. 1998). Mouse models in which the missense mutations of *TECTA* are introduced into *Tecta* may help to explain the molecular pathology of these *TECTA* mutations. Like α -tectorin, mouse β -tectorin encoded by *Tectb* is a major tectorial membrane protein. *Tectb* maps to mouse Chromosome 19 in a region homologous to human 10q25 (Kim et al. 1998) and is a good candidate gene for a deafness locus.

3.6.1 *DFNB21* and *TECTA*

Prelingual, recessive, sensorineural, nonsyndromic deafness *DFNB21* segregating in a single family was reported to be linked to chromosome 11q23–25 (Mustapha et al. 1999). *TECTA* was screened for mutations and affected individuals were found to be homozygous for a donor splice site mutation that truncates *TECTA* by 55%. Heterozygous carriers of this recessive mutation have normal hearing. Mutations of *TECTA* can be either dominant or recessive (Mustapha et al. 1999), indicating that the dominant *TECTA* mutations are unlikely to be null alleles, but rather have a dominant negative mode of action.

3.7 *DFNA9*

Dominant, progressive hearing loss was mapped to 14q12–q13 in a single family and is designated *DFNA9* (Manolis et al. 1996). Two other *DFNA9* families were subsequently identified (Robertson et al. 1998). Hearing loss in these *DFNA9* families begins at 20 to 40 years of age, initially affecting high frequencies and progressing to profound deafness across all frequencies by ages 40 to 50. Affected members of the *DFNA9* family have vestibular dysfunction, but with reduced penetrance and variable expression. Temporal bones of members of a *DFNA9* family contained acidophilic deposits in the cochlear nerve channels, the spiral limbus and the spiral ligament (Khetarpal 1993; Khetarpal et al. 1991). The identity and pathogenic significance of these deposits are unknown.

3.7.1 Human Fetal Cochlear cDNA Library and Data Base

In order to identify the *DFNA9* gene and to provide a candidate gene resource for other investigators, a human fetal cochlear cDNA library was constructed (Robertson et al. 1994). A large number of cochlear EST clones were sequenced. If an EST was not already mapped, it was assigned a map position using a radiation hybrid mapping panel (Skvorak et al. 1999). The human fetal cochlear cDNA database is organized by putative function and chromosomal location, and its URL is http://www.partners.org/pw-cgi/bdml.exe?template=/pweb-view/optional/optional-page.dbml&item_id=7851. This database provides a convenient method to identify cochlear expressed sequence tags (ESTs) that may be positional candidates for *DFNA* and *DFNB* loci based upon their known map positions. Moreover, the map locations of these ESTs are good starting points for genome-wide linkage screens in large families segregating sensorineural hearing loss, once linkage to known deafness loci is eliminated.

3.7.2 Identification of *DFNA9*

One EST in this cochlear database, *COCH*, mapped to 14q12–q13, the location of *DFNA9*. The gene structure of *COCH* was determined and found to have 12 exons with the translation start codon in exon 2 (Robertson et al. 1998). A northern blot analysis indicated that *COCH* is abundantly and specifically expressed in the cochlea and vestibular system (Table 6.4). Affected individuals in each of the three *DFNA9* families were heterozygous for point mutations of *COCH*. All three different point mutations are in a cysteine-rich region and are predicted to cause amino acid substitutions at highly conserved residues of the mouse and chicken *COCH* orthologues (Robertson et al. 1998).

The normal function of *COCH* is unknown and the mechanisms by which mutations in this gene cause hearing loss remain to be elucidated (Fransen et al. 1999). However, information derived from the deduced amino acid sequence of *COCH* gives some idea of what this protein might do in vivo, and whether it is secreted. There are amino acid sequence similarities between *COCH* and a serine protease blood-clotting factor from *Tachypleus tridentatus* (Limulus), raising the obvious question whether *COCH* has serine protease activity. The three point mutations of *COCH* reside in this putative serine protease domain. *COCH* also has two von Willebrand Factor (vWF)-like domains, which have been shown to bind fibrillar collagens, glycoproteins, and proteoglycans. The connection between the acidophilic deposits in temporal bones of affected members of *DFNA9* families and aberrant *COCH* protein remains to be elucidated, but a simplistic hypothesis is that these deposits are composed of *COCH* protein.

In three Dutch families with progressive hearing loss and vestibular impairment that mapped to 14q12–q13, the same dominant allele of *COCH*, P51S, and the same haplotype was observed, suggesting a founder affect in

these families from the Netherlands (de Kok et al. 1999). In other Dutch families and in a Belgian family, the Pro51Ser allele of *COCH* also is responsible for dominantly inherited, progressive vestiulocochlear problems linked to 14q12–13 (Fransen et al. 1999). In some members of these families, the symptoms of episodes of vertigo, fluctuating hearing loss and/or tinnitus is similar to descriptions of individuals with Ménière's disease.

3.8 *DFNA15*

A five-generation Israeli Jewish family segregating autosomal dominant, progressive deafness had twelve affected members. The onset of hearing loss occurred by 18 to 30 years of age and progressed to moderate to severe levels by 40 years of age, with full penetrance (Frydman et al. 2000; Vahava et al. 1998). Markers linked to the known DFNA and DFNB loci were tested for linkage to the hearing loss phenotype in this family. Markers in the *DFNA1* interval at 5q31–q33 showed weak positive linkage at distant recombination fractions from *DFNA1* (Lynch et al. 1997), suggesting that mutations for deafness in the Costa Rican family and the Israeli family were not allelic, but that the two genes are linked. Haplotype analysis defined a 25cM interval for this new locus on 5q, which was designated *DFNA15*. This 25cM critical region was too large to support the use of a positional or functional cloning strategy.

A mouse model suggested a good candidate gene in the region, *POU4F3*. The mouse orthologue, *Pou4f3*, is a member of a family of genes encoding transcription factors, and is necessary for differentiation of sensorineural cells of the auditory system, where it appears to be exclusively expressed (Erkman et al. 1996; McEvilly et al. 1996). A targeted null mutation of *Pou4f3* causes deafness in homozygous mice (Erkman et al. 1996). Mouse *Pou4f3* is located on chromosome 18 in a region of conserved synteny with human 5q. Since the human orthologue, *POU4F3*, mapped to human 5q (Vahava et al. 1998), a mutation screen of *POU4F3* in affected members of the Israeli family was undertaken and revealed an 8bp deletion in exon 2. This frameshift mutation results in a stop codon downstream of the deletion with loss of the high affinity DNA-binding domain from the deduced translation product. This mutation co-segregated with the *DFNA15*-linked haplotype in all affected family members, with the exception of one individual who is a phenocopy (Frydman et al. 2000; Vahava et al. 1998).

The 8bp deletion mutation of *POU4F3* was not present in an older unaffected individuals in the Israeli family, or in 114 unrelated, unaffected control individuals of various Jewish ancestry from the ethnic populations represented in members of the *DFNA15* family. No other *POU4F3* mutant alleles have been found and the 8bp deletion of *POU4F3* has not been found in any other DFNA families. These data suggest that *DFNA15* may not be a major contributor to progressive deafness in early and middle age, but clearly is essential for maintenance of the auditory system in adult life.

Several questions remain about *POU4F3* and hearing loss in humans. Why is the 8bp deletion of human *POU4F3* a dominant mutation, while a *Pou4f3* null mutation in mice is recessive? It is possible that the human mutation is a neomorph resulting in a *POU4F3* protein with a new or altered function. What are the roles of the target genes of *POU4F3* in the development and function of the auditory system? The target genes of *POU4F3* are good candidates for DFNA and DFNB loci that have not yet been identified.

3.9 *DFNB1*

3.9.1 Linkage Analysis and Identification of *DFNB1*

Nonsyndromic recessive deafness (NSRD) segregating in two consanguineous families from Tunisia was mapped by linkage analysis to 13q12 (Guilford et al. 1994a). Many other consanguineous and nonconsanguineous families from different ethnic populations were subsequently shown to also map to *DFNB1* (Brown et al. 1996; Gasparini et al. 1997; Maw et al. 1995; Scott et al. 1995). *DFNB1* therefore appeared to be a major contributor to hereditary deafness in some populations.

3.9.2 *GJB2* Encodes Connexin 26

By virtue of its expression pattern in the inner ear and its location at 13q12, *GJB2* (Gap Junction Protein Beta 2) was examined for mutations cosegregating with NSRD in *DFNB1* families. In two *DFNB1*-linked Pakistani families, two different nonsense mutations of *GJB2* were identified (Kelsell et al. 1997). Subsequently, *GJB2* mutations causing NSRD were identified in a large inbred Israeli-Arab family, a Caucasian family, an Israeli family and a Dominican/Puerto Rican family (Carrasquillo et al. 1997; Scott et al. 1998b).

GJB2 (or connexin 26, *Cx26*) has a simple genomic structure comprising two exons. Exon 1 encodes the 5' untranslated region, while exon 2 encodes the entire 208 amino acid open-reading frame. Because of the small size, a screen for mutations in *GJB2* can be quickly accomplished by SSCP analysis, or more definitively by sequencing the two *GJB2* exons. Many different mutant *GJB2* alleles (<http://www.iro.es/cx26deaf.html>), all located in exon 2 and predicted to inactivate *GJB2*, have been identified in NSRD families from many parts of the world (Carrasquillo et al. 1997; Morell et al. 1998; Scott et al. 1998b).

3.9.3 Connexin Gene Family and Gap Junctions

There are at least thirteen members of the connexin gene family in rodents. Connexins are proteins that comprise gap junctions, which electrophysically couple neighboring cells (Bruzzone et al. 1996). A gap junction is composed

of two half-channels (connexons), each of which is present in either of two adjacent cells. The half-channels then associate to form a gap junction connecting the cells. Each half-channel is composed of six connexin subunits, and different connexins can oligomerize with each other to form a hetero-multimeric half-channel (Bruzzone et al. 1996; Goodenough et al. 1996). The genes encoding proteins that comprise or interact with gap junctions expressed in the auditory system are therefore good candidate genes for DFNB and DFNA loci.

Gap junctions are important for intercellular communication and homeostasis within the inner ear, as they permit cell-to-cell signaling and passage of ions such as potassium (Kikuchi et al. 1995). The endolymph has a high potassium concentration, and potassium flows down a concentration gradient into the hair cells during transduction of the auditory stimulus within the cochlea. It is postulated that potassium is recycled to the endolymph via gap junctions in adjacent support cells and out through fibrocytes of the stria vascularis (Spicer and Schulte 1998; Spicer and Schulte 1996). Pathological mutations of *GJB2* are predicted to disrupt gap junctions comprising defective connexin 26, and thus interfere with the recycling of potassium ions in the inner ear. Since connexin 26 gap junctions probably allow intercellular passage of a variety of other small molecules, altered potassium-ion homeostasis may not completely account for the hearing loss phenotype associated with *GJB2* mutations.

3.9.4 35delG is a Major Contributor to NSRD in Some Populations

One particular allele of *GJB2*, 35delG, also referred to as 30delG, is a major contributor to NSRD in some populations (Denoyelle et al. 1997; Scott et al. 1998b; Zelante et al. 1997). 35delG is a deletion of a single guanine (G) in a string of six Gs beginning at codon 10, and it is presumed to be a null allele. This mutation shifts the reading frame, resulting in a predicted premature chain termination product comprising 12 amino acids, if any at all. The carrier frequency for 35delG in the United States has been calculated from small samples and appears to be about 1/100 to 1/200, but may be higher (Morell et al. 1998; Scott et al. 1998b; Scott et al. 1998a). In French, Spanish and Italian populations, the carrier frequency for 35delG is 1/25 to 1/43 (Denoyelle et al. 1997; Estivill et al. 1998; Zelante et al. 1997), which predicts a high proportion of NSRD is due to this one recessive mutation in these populations. Indeed, 63 to 92% of cases of hereditary nonsyndromic deafness in families originating in Italy, Spain, France, United Kingdom, Tunisia, Lebanon, Australia and New Zealand are associated with the 35delG mutation (Denoyelle et al. 1997; Estivill et al. 1998; Kelsell et al. 1997; Maw et al. 1995; Zelante et al. 1997). Moreover, 35delG accounts for at least 5% of sporadic cases of NSRD. In a cohort of 68 sporadic NSRD cases from the United Kingdom and Belgium, three were homozygous for 35delG and three were heterozygous for 35delG. The second *GJB2* muta-

tion, if it exists in these latter individuals, was not identified (Lench et al. 1998).

3.9.5 167delT Mutation of *GJB2* in Ashkenazi Jews

The limited epidemiological data on the incidence of profound, congenital, hereditary hearing loss among Ashkenazi Jews indicate that the prevalence of NSRD among Ashkenazi Jews is roughly 1 in 2,000. The *GJB2* 35delG carrier frequency is 0.73%, also approximately the same as in the general United States population (Brownstein et al. 1991; Feinmesser et al. 1990). However, a screen of 550 representative, unrelated Ashkenazi Jews demonstrated a carrier frequency of 4.03% for the 167delT mutant allele of *GJB2*, which is rare in the general population (Morell et al. 1998). A conserved haplotype of genetic markers flanking the 167delT mutation was reported. A carrier frequency of 4.76% for just two *GJB2* mutant alleles (4.03% for 167delT and 0.73% for 30delG) would predict a prevalence of one deaf person in 1,765 individuals and could account for much of the recessive hearing loss among the Ashkenazim (Morell et al. 1998). The high carrier frequency of 167delT in the Ashkenazim may have been caused by a previous reduction in population size followed by an expansion, an event referred to as a population bottleneck. A study of idiopathic torsion dystonia predicted a population bottleneck among Ashkenazi Jews about 12 to 13 generations ago (Risch et al. 1995).

3.9.6 Dominant Mutant Alleles of *GJB2*: *DFNB1* and *DFNA3* are the Same Locus

Two alleles of *GJB2* have been reported to be associated with dominant hearing loss. A dominant allele causing nonsyndromic progressive high-frequency hearing loss was mapped to chromosome 13q12 in two families with a total of 30 affected individuals. The locus was designated *DFNA3* (Chaib et al. 1994). In both of these families, a G-to-C transversion was identified at codon 44 of *GJB2*, which is predicted to substitute cysteine for tryptophan (W44C). This dominant allele of *GJB2* was not observed in 380 chromosomes from unrelated, representative individuals, and thus appears not to be a polymorphism (Denoyelle et al. 1998). A second dominant allele of *GJB2*, D66H, has been shown to co-segregate with a syndromic form of deafness known as Vohwinkel's syndrome in three unrelated families, including one large family (Maestrini et al. 1999). Vohwinkel's syndrome is characterized by mutilating keratoderma and sensorineural hearing loss.

Other reports of dominant deafness alleles of *GJB2* have not been so convincing. For example, there is a report of a father and daughter co-segregating profound deafness and palmoplantar keratoderma (PPK) with a heterozygous missense allele of *GJB2*, R75W (Richard et al. 1998). The arginine at residue 75 is conserved among all connexin gene family

members and, therefore, may be important for connexin 26 function. However, demonstration of co-segregation of R75W with deafness and/or PPK in a large family is required in order to establish a causal connection.

M34T is another controversial variant allele of *GJB2* that has been reported to co-segregate with dominant hearing loss in one family with three affected individuals. M34T did not co-segregate with a PPK phenotype, which was also segregating in this same family (Kelsell et al. 1997). Scott and coworkers (1998a) subsequently reported a family with three normal-hearing members who were each heterozygous for M34T, leading them to suggest that it is not a dominant mutant allele. We have also observed the M34T allele in a nonsyndromic recessive deafness kindred where it did not co-segregate with the hearing-loss phenotype, indicating that it is likely to be a polymorphism (Griffith et al. 2000a).

The functional significance of the M34T mutation has been addressed by electrophysiologic approaches. The channel-forming ability of connexin 26 subunits containing the M34T mutation has been examined *in vivo* by coupling *Xenopus laevis* oocytes or HeLa cells expressing the connexin 26 subunits and measuring intercellular conductance (Martin et al. 1999; White et al. 1998). The wild-type allele of *GJB2* produced robust conductances, indicating high levels of channel activity (Martin et al. 1999; White et al. 1998). On the other hand, the M34T product and a well characterized recessive allele of *GJB2*, W77R (Kelsell et al. 1997; Scott et al. 1998b), both failed to induce significant intercellular conductance. Moreover, intercellular conductance was significantly reduced in coupled *Xenopus* oocytes co-expressing the M34T allele and the wild-type *GJB2* allele, indicating a dominant negative effect of M34T (White et al. 1998). The validity of extrapolating these results of the coupled *Xenopus* oocyte or HeLa cell assay to the *in vivo* effects of *GJB2* variant alleles on auditory function has not been established. Co-segregation of these alleles with dominant deafness in large families is required in order to conclude that they are indeed dominant mutant alleles.

There are reports of hearing-impaired individuals in which only one of two mutant *GJB2* alleles in a presumed compound heterozygote could be found. In some families with nonsyndromic recessive deafness, many of which are too small for linkage analysis, only one recessive mutant allele of *GJB2* was identified (Denoyelle et al. 1997; Scott et al. 1998b). Cohn and coworkers (1999) found only one mutant allele of *GJB2* in 6 of 68 families with NSRD. There are several reasons the partnering *GJB2* mutant allele might not have been found: The second allele may be in a region of *GJB2* that was not screened for mutations, including the cis-acting regulatory elements of the promoter, the 5' untranslated region (UTR), or the intron.

A parsimonious explanation for not finding the second mutant recessive allele of *GJB2* in some individuals with NSRD is that it is not present and *GJB2* mutations are not the cause of the hearing loss. Some populations

have a 3% carrier frequency for the 30delG mutant allele of *GJB2* and, therefore, one in 33 people in the general population, including individuals with hearing loss, will be a 30delG carrier. The detection of a heterozygous 30delG mutation in an individual or a small family with NSRD may simply be coincidental (Estivill et al. 1998).

Another possibility is that the second mutation may be in another gene, and the combined action of the two nonallelic mutations gives rise to a mutant phenotype. Other connexin genes would be good candidates for harboring the “missing deafness allele,” since it is known that gap junctions can be heteromultimeric complexes comprising different connexin family members such as connexin 26, connexin 32, connexin 46 and connexin 50 (Bruzzone et al. 1996).

3.9.7 Hearing Phenotype in Families with *GJB2* and *GJB3* Mutations

There is considerable variation in the hearing phenotype among and within *DFNB1* families. The phenotype may be a congenital moderate to profound hearing loss, or it might be a progressive loss in some families. Such variation is indicative of the existence of a modifier gene(s) (Carrasquillo et al. 1997; Denoyelle et al. 1997; Morell et al. 1998; Scott et al. 1998b). The same modifier genes could also account for the nonpenetrant status of carriers of the R180X mutant allele of *GJB3* (Scott et al. 1998a; Xia et al. 1998). A large inbred population with NSRD and significant phenotypic variation among individuals homozygous for the same allele of *GJB2* (or *GJB3*) could be useful for mapping and identifying such modifier genes (Carrasquillo et al. 1997).

Surprisingly, there are a few individuals reported to be homozygous for the 35delG mutation (presumably devoid of functional connexin 26) who exhibit mild or progressive hearing loss (Cohn et al. 1999). These observations indicate that there must be a compensatory pathway in some individuals for the loss of connexin 26 function in the auditory system. Manipulation of this compensatory mechanism is one potential approach to the development of pharmacologic therapy for *DFNB1* individuals with hearing loss. The considerable variation in hearing loss within and between families associated with *GJB2* mutant alleles also poses a challenge to genetic counselors and medical geneticists attempting to inform their patients of potential risks.

3.9.8 Animal Models for *GJB2*

Mapping and identifying the genes modifying the *GJB2* (or *GJB3*) phenotype might be more easily achieved in the mouse, where large breeding crosses can be used to generate many meioses, which facilitate gene localization and identification. However, a homozygous targeted intragenic deletion (null allele) of *Gjb2* in the mouse is reported to result in embryonic lethality 10 days post-coitum (Gabriel et al. 1998). This is in contrast to the

effects of homozygous null alleles of *GJB2* in humans, in whom *GJB2* does not appear to be essential for viability or development. It is possible that mutant mouse missense alleles may not cause a lethal phenotype in their mouse orthologues, in which case a search for modifying genes would be possible. These and other mouse models would also be useful for electrophysiologic and histopathologic evaluations of the effects of connexin mutations in the auditory system, studies that are difficult to carry out in humans.

3.10 *DFNB2, DFNA11 and USH1B are the Same Locus*

Nonsyndromic recessive deafness associated with partially penetrant vestibular dysfunction was initially mapped to 11q by linkage analysis in a consanguineous family from South Tunisia (Guilford et al. 1994a). The location was subsequently refined by homozygosity mapping to 11q13.5 (Guilford et al. 1994a), and the locus was designated *DFNB2* (Fig. 6.1 and Table 6.2). Since the Tunisian family is consanguineous, markers in the region closely flanking *DFNB2* are expected to be homozygous by shared descent from an ancestral allele (Lander and Botstein 1987). All 24 affected individuals of this family were homozygous for the *DFNB2*-linked haplotype consisting of seven markers across the 6-cM interval on 11q13.5. Heterozygosity for markers flanking this interval demarcated the *DFNB2* critical region. Thirty-one of 32 unaffected members of the family had one or no copies of the *DFNB2*-linked haplotype, whereas one normal-hearing person who was twenty-five years old was homozygous for the *DFNB2*-linked haplotype. The possibility of reduced penetrance of *DFNB2* is supported by the observation of highly variable severity (ranging from mild to profound SNHL) and age of onset (ranging from congenital to onset at 16 years of age) of hearing loss in the affected members of the Tunisian kindred.

3.10.1 *Shaker 1* is the Orthologue of *DFNB2, DFNA11* and *USH1B*

Usher syndrome is a clinically and genetically heterogeneous disorder characterized by autosomal recessive transmission of sensorineural hearing loss and retinitis pigmentosa (see Section 5.4.2). One particular subtype of Usher syndrome, type 1B, was mapped to human chromosome region 11q13.5 (Kimberling et al. 1992). The known genes located on 11q13.5 share conserved linkage with their homologues on part of mouse chromosome 7 containing the interval to which the *shaker 1* (*sh-1*) mutation maps (Evans et al. 1993). Homozygous *sh-1* mice demonstrate a phenotype of circling behavior and neurosensory deafness, therefore presenting an excellent candidate gene for hereditary deafness disorders in humans (Lyons and Searle 1989).

3.10.2 *Shaker 1* Encodes Unconventional Myosin VIIa

Shaker 1 was identified by a physical map-based strategy (positional cloning) and shown to encode an unconventional myosin designated *Myo7a*. Myosin genes are subdivided into conventional myosins (e.g., myosin class II of muscle and nonmuscle cells) and unconventional myosins (classes I, III–XV) (Mermall et al. 1998; Wang et al. 1998). Myosins are motor-proteins that bind cytoskeletal actin and hydrolyze ATP to produce force and movement (Mermall et al. 1998). Myosin genes have an evolutionarily conserved motor domain that is usually located at the amino terminus, and a C-terminal tail domain that is divergent between different myosin classes (Fig. 6.4). The functions of some unconventional myosins are just now becoming known, and the existing data indicate a role for these molecular motors in intracellular transport along actin filaments. Unconventional myosins are involved in the processes of endocytosis, regulation of ion channels, localization of calmodulin, movement of vesicles and particles in the cytoplasm, determinant localization and anchoring inner ear cell stereocilia (Baker and Titus 1998; Mermall et al. 1998; Titus 1998).

MYO7A mutations were found in two small Chinese families (Liu et al. 1997b) and in the original *DFNB2* family from Tunisia segregating nonsyndromic recessive deafness (Weil et al. 1997) (Fig. 6.4). An A-to-G transition in the last nucleotide of exon 15, encoding part of the *MYO7A* motor domain, was found in the Tunisian family. This mutation is predicted to result in substitution of isoleucine for methionine (M599I) (Weil et al. 1997). However, based on its position as the last nucleotide of exon 15, this mutation could exert a deleterious effect by affecting the splicing efficiency of *MYO15* mRNA. Compound heterozygosity or homozygosity for three additional *MYO7A* mutations, R244P, a splice-acceptor-site mutation, and a frameshift mutation (Val1199insT[FS]), co-segregated with NSRD in the two small families from China (Liu et al. 1998; Liu et al. 1997b). Establishing a convincing argument for a causal connection between the missense mutation R244P and hearing loss is difficult in a small family. However, the causality of this mutation is supported by the observation that *sh-1^{6J}* is also a substitution of proline for arginine at the equivalent position in the myosin motor (Mburu et al. 1997).

3.10.3 *DFNA11* is the Same Locus as *DFNB2*

Dominant, progressive, nonsyndromic hearing loss was mapped to 11q12.3-q21 in a Japanese family and designated *DFNA11* (Tamagawa et al. 1996). This is the same region to which *DFNB2* and Usher's syndrome type 1B were mapped. Hearing loss in the *DFNA11* family is postlingual and progresses to a moderate loss by 60 years of age. All eight affected members of the *DFNA11* family have a 9-bp deletion of exon 22 of *MYO7A*, which removes three amino acids, but otherwise maintains the correct reading frame. Exon 22 encodes a coiled-coil domain that allows myosin VIIA to

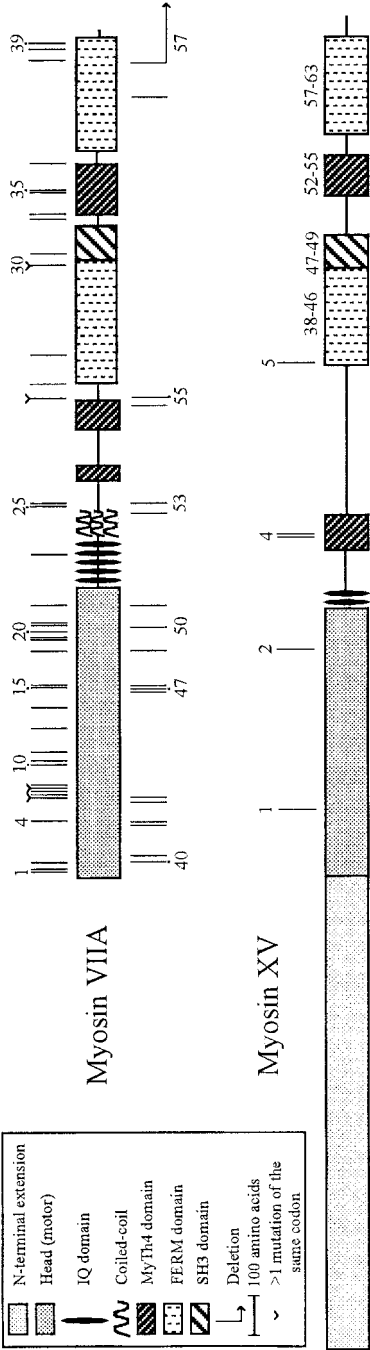


FIGURE 6.4. Schematic drawings of the longest reported isoforms of myosin VIIA and myosin XV depicting the conserved motor domain, the neck containing one or more IQ motifs, the divergent tails and the unique N-terminal domain of myosin XV. The amino acid sequences of myosins VIIA and XV are based on accession numbers U39226.1 and AF051976, respectively. The gene structures for myosins VIIA and XV are from Kelley et al. 1997 and Liang et al. 1999. The tail domains of myosins VI and VIIA contain a short segment predicted to form a coiled-coil that may allow for dimerization to a two headed molecule. SH3 domains, which are thought to interact with proline-rich sequences, have been found in other myosins, as has the MyTh4 (myosin tail-homology) domain, which is of unknown function. The FERM (band 4.1-ezrin-moesin-radixin motif, formerly called a Talin domain) is also found in other myosins as well as other cytoskeletal proteins, and may mediate cytoskeletal protein interactions. Vertical lines above the schematic diagrams are missense or nonsense mutations of myosins VIIA and XV. Vertical lines below the schematic indicate small deletions, insertions or splice-site mutations. For mutations of myosin VIIA, a "U," "A" or "B" after the mutation number indicates *USH1B*, *DFNA11* or *DFNB2*, respectively. 1U (L16X), 2U (G25R), 3U (C31X), 4U (R150X), 5U (R212H and R212C), 6U (G214R), 7U (Q234X), 8U (R241S), 8 *sh1*⁶¹ (R241P), 9B (R244P), 10U (R302H), 11U (E314X), 12U (Y333X), 13U (A397D), 14U (E450Q), 15 *sh1* (R502P), 16U (P503L), 17B (M599I), 18U (C628X), 19U (R634X), 20 atypical USH (L651P cpd. het. w/ R1602Q), 21U (R666X), 22U (R669X), 23 *sh1*^{4626SB} (Q720X), 24U (A826T), 25U (G955S), 26U (E960X), 27U (R1240Q and R1240E), 28U (A1288P), 29U (R1343S), 30U (R1602Q) and atypical USH (R1602Q cpd. het. w/ L651P), 31U (A1628S), 32U (Y1719C), 33U (R1743W), 34U (Q1798X), 35 *sh1*^{265SB} (F1800I), 36U (R1861X), 37U (G2137E), 38U (G2163S), 39 *sh1*^{336SB} (C2182X), 40B (IVS3-2), 41U (75delIG), 42U (IVS5 + 1), 43U (120delC), 44 *sh1*^{4948SB} (IVS6nt + 2T → A), 45U (ΔD218-1219), 46U (468 + Q), 47U (IVS13-8), 48U (532delA), 49 *sh1*^{816SB} (IVS16nt-2), 50U (IVS18 + 1), 51U (724delC), 52A (Δ886-888), 53U (IVS24-21), 54B (V1199 + T), 55U (IVS29 + 2), 56U (2065delC), 57U (>2kb deletion); myosin XV.1 (G1358S), 2 *sh2* (C1775Y), 3 (N2111Y), 4 (I2113F), 5 (L2601X). The original description of these myosin VIIA and myosin XV mutations are in the references cited in Table I of Friedman et al. 1999 from which this figure was taken with modifications and in Cuevas et al. 1998 and Mburu et al. 1997.

dimerize with itself (Weil et al. 1997), and therefore this allele may be dominant because its gene product can interact with wild-type myosin VIIA to form a defective dimer (Fig. 6.4). Some functional dimers would be predicted to form between wild-type myosin VIIA polypeptides, leading to a milder hearing loss phenotype in comparison with the profound congenital deafness associated with homozygosity for recessive alleles causing total loss of functional MYO7A.

3.10.4 Multiple Alleles of *MYO7A*

There are over fifty mutations of *MYO7A* that give rise to a spectrum of phenotypes (Adato et al. 1997; Levy et al. 1997; Liu et al. 1998; Weston et al. 1996). Mutations that cause nonsyndromic recessive deafness, *DFNB2*, and Usher syndrome 1B are distributed across the coding region of *MYO7A* (Fig. 6.4). Except for the *DFNA11* mutation in the coiled-coil domain of *MYO7A*, there is no obvious pattern of mutation location or mutation type, (missense, nonsense, frameshift, etc.) that would predict whether a particular allele will cause hearing loss that is nonsyndromic, or hearing loss that is associated with retinitis pigmentosa. If phenotypic variability is not entirely attributable to allelic heterogeneity of *MYO7A*, is it the genetic background that is contributing to the pleiotropic phenotype? This question is clinically important because identification of the modifier gene(s) that permits or prevents blindness should be helpful in devising therapeutic strategies to forestall the progressive blindness associated with type 1B Usher's syndrome.

3.11 *DFNB3*

Mapping genes for recessive hearing loss can be more easily accomplished in remote populations where there is a probable founder effect. *DFNB3* was first mapped in the village of Bengkala on the island of Bali, Indonesia (Friedman et al. 1995; Winata et al. 1995). There were 24 deaf males and 24 deaf females living in this village who shared a unique sign language with one another and with the other 2,206 hearing members of the village (Friedman et al. 2000; Hinnant 2000). Affected individuals cannot detect sound at levels as high as 90 dB at any tested frequency. Formal vestibular testing has not been performed, but some affected individuals describe balance problems and dizziness when their eyes are closed, suggesting the presence of vestibular dysfunction.

In a genome-wide screen, *DFNB3* was one of the first human disease genes to be localized using a homozygosity mapping strategy (Friedman et al. 1995). The deafness segregating in Bengkala was mapped to the pericentromeric region of chromosome 17 (Friedman et al. 1995), and the location of *DFNB3* was subsequently refined to a 4 to 5 cM region on chro-

mosome 17p11.2 (Fig. 6.1) (Liang et al. 1998). Two consanguineous families from India with profound congenital deafness were also found to be linked to genetic markers in the 17p11.2 region, suggesting that there are multiple alleles of *DFNB3* (Liang et al. 1998).

3.11.1 Identifying *DFNB3* by Functional Cloning of Mouse *Shaker 2*

The map position of *DFNB3* on human 17p11.2 predicted a homologue, *shaker 2* (*sh-2*), in the syntenic region of mouse chromosome 11 (Friedman et al. 1995). Homozygous *sh-2* mice are deaf and exhibit circling behavior. *shaker 2* was mapped in a 500-meiosis cross to a critical region of 0.2 cM (~400 to 800 kb of DNA) (Liang et al. 1998). A physical map of this region was constructed using Bacterial Artificial Chromosomes (BACs). In Sally Camper's laboratory, each BAC was individually injected into the pronucleus of homozygous *sh-2* fertilized eggs, and the eggs were then transferred to a foster mother. One transgenic offspring did not circle, and responded to sound with a normal Preyer's reflex. This mouse had been injected with BAC425p24. The mouse was mated and correction of the *shaker 2* phenotype was correlated with transmission of BAC425p24 in the germline of his offspring. The location of *sh-2* was thus refined from ~400–800 kb to just 140 kb, the insert size of BAC425p24 (Probst et al. 1998).

The DNA sequence of BAC425p24 was analyzed using two computer programs that detect probable protein-coding regions of genes (exons) (GRAIL, <http://compbio.ornl.gov/Grail-bin/EmptyGrailForm> and GENSCAN, <http://CCR-081.mit.edu/GENSCAN.html>). These analyses predicted two genes encoded in BAC425p24, one of which is a novel unconventional myosin that was designated myosin XV (*Myo15*). It was already known that mutations of other unconventional myosins could cause hearing loss (Adato et al. 1997; Avraham et al. 1997; Weil et al. 1997) and, therefore, *Myo15* was a strong candidate for *sh-2*.

3.11.2 The *Shaker 2* Locus Encodes *Myo15*, an Unconventional Myosin

Myo15 has 66 exons with several splice isoforms (Liang et al. 1999) (Fig. 6.4). Each of the 66 exons was screened for mutations in the two known alleles of *shaker 2*: *sh-2* and *sh-2'*. The *sh-2* allele has a G-to-A transition in the motor domain of myosin XV that substitutes tyrosine for a highly conserved cysteine (Cope et al. 1996; Probst et al. 1998). The *sh-2'* allele was shown to have a deletion of the last 6 exons of *Myo15* (Anderson et al. 2000).

Some clues about the role of myosin XV in the inner ear can be obtained from light- and electron-microscopic studies of tissues from *shaker 2* and *shaker 2'* mutant mice. Hair cells were present, but the stereocilia on the inner and outer hair cells were very short, approximately 1/10 of the normal length (Probst et al. 1998). The stereocilia of the inner hair cells are essen-

tial for transducing the physical stimulus of sound within the cochlea to an electrical stimulus transmitted to the auditory nerve. The very short stereocilia may explain the lack of hearing in *shaker 2* mice.

3.11.3 Mutations of Human *MYO15* Cause Deafness

Identification of *shaker 2* as *Myo15* triggered a search for *MYO15* mutations in *DFNB3* families. The structure of *MYO15* was determined by sequencing three overlapping cosmid clones corresponding to approximately 90kb of contiguous genomic DNA. The predicted 66 exons of *MYO15* were identified and confirmed by sequencing human *MYO15* cDNA synthesized from human fetal inner ear, adult brain and pituitary gland mRNA (Liang et al. 1999). Genomic DNA encoding *MYO15* was sequenced from probands of the three *DFNB3* families previously described. Two missense mutations and one nonsense mutation in *MYO15* were identified (Fig. 6.4), and each of the three mutations co-segregated with the deafness phenotype in the three *DFNB3* families (Wang et al. 1998).

Many questions remain about the roles of myosin XV, as well as myosin VI and myosin VIIA, in the auditory and vestibular systems and in the epidemiology of deafness. What are the other proteins that interact with myosin XV and/or are transported within the cell by myosin XV? The identification of myosin XV protein partners will be helpful in illumination of the function of myosin XV, and they will be good candidates for other hearing impairment genes. Surprisingly, myosin XV is abundantly expressed in the anterior pituitary gland, although there is no obvious pituitary phenotype in deaf individuals homozygous for mutant alleles of *MYO15*. It is possible that there is functional redundancy for myosin XV in the pituitary gland, where other proteins may be able to compensate for the loss of functional myosin XV.

Although the phenotype of *shaker 2* suggests a role for myosin XV in maintaining the cytoskeleton of stereocilia in hair cells, nothing is known with confidence about the specific function of this novel unconventional myosin. However, *Myo15* in inner ear hair cells is immunolocalized to the cuticular plate and to the tops of stereocilia (Liang et al. 1999). Based on its location at the top of hair cell stereocilia by electron microscopy, we speculate that *Myo15* is part of the adaptation motor that is responsible for regulating tip link tension and gating hair cell mechanotransduction channels (Bechara Kachar, personal communication).

3.12 *DFNB9*

In a large Lebanese family, a novel gene responsible for recessive, sensorineural, nonsyndromic deafness was mapped to 2p22–23 (Chaib et al. 1996a). Deafness was profound and congenital. Using a candidate gene approach, a mutation in *OTOF* was identified (Yasunaga et al. 1999). *OTOF*

encodes a protein, otoferlin, that is expressed predominantly in organ of Corti postnatal inner hair cells. The deduced sequence is predicted to encode three C2-like domains and suggests that otoferlin may be involved in synaptic vesicle trafficking.

3.13 *DFN Loci*

About 1 to 3% of childhood nonsyndromic recessive deafness (NSRD) is due to mutant alleles of genes that are sex- or X-linked (Fraser 1965; Reardon et al. 1992). Sex-linked NSRD is clinically and genetically heterogeneous with five loci, *DFN1*, *DFN2*, *DFN3*, *DFN4* and *DFN6*, described to date (Table 6.3). The nomenclature for sex-linked hearing loss is confusing because gene symbols were assigned prior to genetic mapping and, in the case of *DFN1*, before clinical evaluations revealed that affected members have a form of syndromic hearing loss now referred to as Mohr-Tranebjaerg syndrome, MTS.

The X-linked loci causing nonsyndromic hearing loss are discussed in numerical order, including *DFN1*. To date, mutant alleles of *DFN1*, *DFN3* and *DFN6* are associated with more mild hearing loss, in comparison with mutant alleles of *DFN2* and *DFN4* that cause profound childhood deafness.

3.14 *DFN1 is Mohr-Tranebjaerg Syndrome*

A Norwegian family was described that segregated X-linked nonsyndromic, sensorineural, postlingual, progressive deafness. Because of the obvious pattern of transmission from mothers to sons, the locus was assigned to the X chromosome in this family and designated *DFN1* (Mohr and Mageroy 1960). Clinical re-evaluation of the affected members of this family in 1995 revealed that progressive hearing loss was one of the first presenting neurological deficits of a more generalized phenotype including progressive cortical blindness, dystonia, spasticity and mental deterioration. As a result, *DFN1* was reclassified as syndromic deafness, but still designated *DFN1* and called Mohr-Tranebjaerg syndrome, MTS (Tranebjaerg et al. 1995).

A clue to the identity of the *DFN1* gene came from a patient with a contiguous deletion syndrome composed of X-linked immunodeficiency agammaglobulinemia (XLA OMIM 300300), dystonia, and progressive sensorineural hearing loss. The deletion was found to encompass five genes. One of these genes is *BTK*, in which point mutations cause XLA (Vetrie et al. 1993). The *DDP* (deafness dystonia peptide) gene was also deleted in this patient and was a candidate for MTS. The coding region of *DDP* is divided into two exons encoding a 97 amino acid peptide of 11 kDa. Two different frameshift mutations in *DDP* were identified in the Norwegian *DFN1* pedigree and in an additional family segregating only deafness and dystonia (Jin et al. 1996).

DDP is expressed at high levels in the fetal and adult brain, as well as in many other cell types. Insight into the role that *DDP* plays in vertebrate neurological development and in auditory function has come from characterization of the *DDP* orthologues (Tim8p, Tim9p, Tim10p, Tim12p and Tim13p) in the yeast *Saccharomyces pombe*. Tim8p shares 49% identity and 60% similarity over 63 amino acids with human *DDP*. *DDP*, like Tim8p, is a nuclear-encoded mitochondrial protein that mediates import of metabolites from the cytoplasm into the mitochondrial inner membrane (Koehler et al. 1999).

3.15 *DFN2*

DFN2 was mapped in a large British family segregating X-linked profound congenital sensorineural hearing loss. The deafness phenotype in this family was first tested for linkage to markers near *DFN3* and *DFN4*, since these loci had already been mapped. There were meiotic recombinations of the hearing loss phenotype with *DFN3*-linked markers in affected males and carrier females, some of whom had mild to moderate hearing loss. *DFN3* could thus be excluded as the causative locus and, similarly, hearing loss was shown to not be linked to the *DFN4* interval or to the X-linked Alport's syndrome locus (*COL4A5*, Fig. 6.1) (Tyson et al. 1996). A screen of X-linked markers was undertaken and a new locus, *DFN2*, was mapped to Xq22 in this family. *DFN2* is yet to be identified.

3.16 *DFN3*

Many families with X-linked progressive mixed hearing loss have been described. Affected individuals typically have fixation of the stapes footplate, and a brisk flow of perilymphatic fluid from the vestibule (known as a "perilymphatic gusher") is encountered when the stapes footplate is opened during the stapedectomy procedure to correct the conductive hearing loss (Cremers et al. 1985; Nance et al. 1971). Families segregating X-linked sensorineural hearing loss without a conductive component also map to *DFN3*. Computed tomography (CT) scanning of temporal bones and inner ears have revealed associated osseous defects, which have been proposed to be pathognomonic for *DFN3* mutations (Bach et al. 1992; Phelps et al. 1991), and serve as a warning to surgeons contemplating corrective stapes surgery.

The mixed hearing loss was mapped to Xq21.1 by linkage analyses, cytologically detectable deletions and overlapping microdeletions, and this locus was designated *DFN3* (Huber et al. 1994). Unlike many other regions of the X-chromosome, cytologically detectable nullisomy for Xq21 is compatible with life and is usually associated with hearing loss. However, there are a few normal-hearing patients with a deletion of Xq21, an inconsistency

that remains unexplained (Bach et al. 1992). Perhaps some Xq21 deletions are accompanied by a duplication that includes the *DFN3* gene.

A candidate gene for *DFN3* was identified on the basis of conserved synteny between the human and mouse genetic maps. Murine *Brain 4* is an intronless gene that encodes the transcription factor *Pou3F4* and maps to a region that predicted a human homologue, *POU3F4*, at Xq13-q22 (Douville et al. 1994). *Pou4f3*-deficient mice are fertile and have normal vestibular function. However, they show a reduced endocochlear potential, structural alterations of the cochlear spiral ligament fibrocytes and are profoundly deaf (Minowa et al. 1999).

POU3F4 mutations were detected in some patients with *DFN3*-linked hearing loss, as well as in families with X-linked hearing loss that were too small to map the phenotype (Bitner-Glindzicz et al. 1995). However, not all individuals with the *DFN3* phenotype that map to *DFN3*, either by linkage or by deletion analyses, have point mutations in the open reading frame (ORF) of *POU3F4*. Some *DFN3* patients have mini-deletions more than 400kb upstream of *POU4F3*. Deletions approximately 900kb upstream of *POU4F3* also are associated with the *DFN3* phenotype (de Kok et al. 1996). The genomic DNA of one *DFN3* patient shows a complex chromosomal rearrangement involving a paracentromeric inversion 320kb from *POU3F4* and a 150 to 170kb duplication proximal to *POU3F4*, but the coding region of *POU3F4* is intact. One interpretation is that a *POU3F4* regulatory control element is located 400kb upstream of the *POU3F4* ORF and is separated from *POU3F4* by the inversion event (de Kok et al. 1995). Alternatively, the inversion/duplication event may alter higher order chromatin structure and thus disrupt *POU3F4* expression (Kleinjan and van Heyningen 1998).

3.17 *DFN4*

DFN4 was mapped to chromosome Xp21.2 in a family in which males are born deaf and carrier females show a delayed onset, mild to moderate sensorineural hearing loss. Haplotype analysis of this one family indicated that the *DFN4* critical region partially overlaps the Duchenne Muscular Dystrophy (*DMD*) gene. *DMD* spans 2.5 million base pairs of DNA and encodes dystrophin, a 427kDa protein, as well as smaller *DMD* isoforms such as a 260kDa dystrophin required for normal retinal electrophysiology (D'Souza et al. 1995). The *DFN4* gene is either closely linked to *DMD*, is nested in one of the large introns of *DMD*, or is a mutant allele of *DMD* (Lalwani et al. 1994).

Additional *DFN4* families were sought to help distinguish between these three possibilities. Pfister (1998) described a Turkish *DFN4* family in which one presumed carrier female (individual II-10) with mild hearing loss has a meiotic breakpoint that refines the map location of *DFN4* within the

DMD locus. However, II-10 has only an unaffected son, which does not genetically confirm her carrier status (Pfister et al. 1998).

Unlike *DMD* patients, *DFN4*-affected individuals had no gross rearrangements of the 79-exon dystrophin gene in Southern blot and PCR analyses. *DFN4* males show no clinical characteristics of Duchenne muscular dystrophy, and there are no data indicating that patients with *DMD* or Becker muscular dystrophy have sensorineural hearing loss. However, the *mdx* mouse has a nonsense mutation in exon 23 of *Dmd* and sensorineural hearing loss as measured by ABR analysis (Raynor and Mulroy 1997). A structural role for dystrophin in the auditory system is consistent with its expression in the organ of Corti, where it has been localized by immunohistochemistry to the lateral membrane and cuticular plate of the hair cells. *DMD* therefore remains a candidate for *DFN4* (Dodson et al. 1995).

3.18 *DFN6*

A clinically distinct X-linked progressive, nonsyndromic, sensorineural deafness was described in a five-generation Spanish family with ten affected males. Hearing loss in this family did not show linkage to the other *DFN* loci, and was then mapped by linkage analysis to Xp22 and refined by haplotype analysis to a 15 cM interval (del Castillo et al. 1996). Males begin showing a high-frequency loss at age 5 to 7 years that progresses to profound deafness. Seven of the ten carrier females have a moderate hearing loss with the onset delayed until 40 years of age. The affection status of other family members who are now under the age of 5 years may eventually be definitively established, at which time their genotypes may allow further refinement of the map position of *DFN6* and the eventual identification of this gene.

3.19 Evidence for Digenic Inheritance of Nonsyndromic Hearing Loss

Mutations of a single gene can explain the hearing loss in the majority of *DFN*, *DFNA* and *DFNB* families that have been ascertained. However, there are two reports of familial hearing loss that suggest a pattern of inheritance involving two loci (digenic inheritance). The first example is a small consanguineous family with three affected and three unaffected siblings. A genome-wide screen identified two regions of homozygosity-by-descent shared by the three affected children on 3q21.3-q25.2 (Lod = 2.78) and 19p13.3-p13.1 (Lod = 2.78). The authors speculated that homozygosity for two nonallelic recessive mutations may account for the profound congenital deafness in this family (Chen et al. 1997).

The second possible example of digenic inheritance is a Swedish family segregating nonsyndromic, postlingual, progressive sensorineural hearing loss. Initial pedigree analysis suggested that a single autosomal dominant mutation could account for the observed inheritance of hearing loss, which

showed variable severity and variable age of onset ranging from approximately 7 to 30 years of age. A genome-wide screen for linked polymorphic markers yielded a peak lod score of 3.87 for marker D11S4171, with the recombinants defining a 12-cM critical region that includes the *DFNA12* locus on 11q22-q24 (Fig. 6.1). However, a peak multipoint lod score of 2.69 was also found for markers linked to *DFNA2* at 1p32 in the same family (Balciuniene et al. 1998). Since a lod score of ≥ 3.0 is considered minimal statistical evidence of linkage, the observation of weak linkage to 1p32 is provocative, but not statistically significant. It is possible that the markers near *DFNA2* are showing weak linkage by chance alone. Alternatively, these results may indicate that two different DFNA genes are segregating in this family, each of which independently causes hereditary hearing loss. It is also possible that there is an interaction or additive effect of mutations at *DFNA2* and *DFNA12* that cause the observed phenotypic heterogeneity in this family.

This latter hypothesis is consistent with the observation that, with a single exception, the most severely affected family members had haplotypes linked to both *DFNA2* and *DFNA12*. However, individuals with milder hearing loss and later mean age of onset had haplotypes linked either to *DFNA2* or *DFNA12*, but not to both (Balciuniene et al. 1998). Definitive proof of digenic inheritance of the hearing loss phenotype in this Swedish family will require identification of two non-allelic-dominant mutations in the most severely affected individuals: one in *DFNA12* and one in *DFNA2* or a closely linked locus.

3.20 Summary of the Molecular Genetics of *DFN*, *DFNB* and *DFNA* Loci

Generalizations about the nonsyndromic hearing loss loci have emerged from clinical characterization of families with NSRD, and from mapping and identifying these genes.

(1) The issue of genetic heterogeneity is usually circumvented in studies of hereditary hearing loss in consanguineous families and geographical and cultural isolates, since they are often segregating a single mutant allele for hearing loss. Affected individuals are likely to be homozygous for the same alleles of the disease gene and, just as importantly, the same alleles of closely linked markers (Friedman et al. 1995; Jaber et al. 1998; Sheffield et al. 1998). The size of the interval showing linkage disequilibrium with the phenotype will vary inversely with the number of generations since the mutation was introduced.

(2) The mapping of over 30 DFNA and 30 DFNB loci provides abundant experimental data to support the claim that hearing loss is genetically heterogeneous. Ongoing studies indicate that there are many more DFNA and DFNB loci to be mapped, since there are still additional families seg-

regating hereditary hearing loss which is not linked to known phrasing hearing loss loci.

(3) There are now two examples of mutations of the same gene that cause both syndromic and nonsyndromic hearing loss. Alleles of *MYO7A* (*DFNB2*, *DFNA11*) are associated with nonsyndromic sensorineural hearing loss, as well as type 1B and atypical Usher syndrome phenotypes (Liu et al. 1998) (see Section 5.4). Moreover, mutations of *PDS* can cause Pendred syndrome, as well as nonsyndromic recessive deafness, *DFNB4* (see Section 5.2). A cytogenetic map of nonsyndromic and syndromic loci associated with hearing loss is shown in Figure 6.1. When the genetic map locations for a nonsyndromic hearing loss locus and a syndromic hearing loss locus overlap, it is worth considering the possibility that different alleles of the same gene may be responsible for both forms of hereditary hearing impairment.

(4) There are also both dominant and recessive mutant alleles of *GJB2*, *MYO7A* and *TECTA*. The historical distinction between *DFNA* and *DFNB* loci will probably continue to grow more obscure as additional alleles of these genes are identified. "Dominance and recessiveness are not properties of genes per se but the result of the action of the genetic locus in question . . ." (Rieger et al. 1991).

(5) Two of the six *DFNB* loci identified so far, *DFNB2* and *DFNB3*, encode unconventional myosins *MYO7A* and *MYO15*, respectively. The functions of these two molecular motors in the auditory system, as well as those encoded by *MYO6* (Avraham et al. 1995) and *MYO1 β* (Gillespie and Corey 1997), are actively being studied, but remain enigmatic.

(6) Mouse hearing loss loci have been instrumental in identifying the human orthologues. The identification of the mouse *shaker1* and *shaker2* genes greatly facilitated the identification of *DFNB2* and *DFNB3*, respectively. Saturation mutagenesis screens and mapping studies of new hearing loss mutations in the mouse should further accelerate discovery of the human counterparts. Moreover, once a human gene for hearing loss is identified, the mouse provides an excellent model system for studying the spatial and temporal expression profiles of these genes, as well as the phenotypic effects of the corresponding mouse mutations (Steel and Bock 1983).

(7) With the exception of *DFNB1* (*GJB2*), for which epidemiological data is emerging, little is known about the contribution made by each *DFN*, *DFNA*, and *DFNB* locus to hereditary hearing loss worldwide.

4. Otosclerosis

Otosclerosis (MIM 166800) is a common cause of hearing loss in the adult Caucasian population. It is characterized by one or more histologic foci of progressive endochondral bone sclerosis within structures of the otic

capsule. Approximately 8% of temporal bones from the Caucasian population show evidence of histologic otosclerosis, although only 1% of the Caucasian population manifests hearing loss associated with clinical otosclerosis (Altmann et al. 1967). The same study reported a lower prevalence of histologic otosclerosis in black, Asian, and American Indian populations (Altmann et al. 1967).

The hearing loss is typically conductive, but may progress to a profound mixed loss in later stages of the disease. The conductive component is caused by fixation of the stapes footplate in the oval window by otosclerotic tissue. The etiology of the sensorineural loss, termed "cochlear otosclerosis," is not well understood, but has been postulated to be caused by direct mechanical effects, or by metabolic or vascular factors associated with the otosclerotic process within the cochlea. Fortunately, the conductive hearing loss may be reduced or eliminated by modern surgical techniques that re-establish efficient sound transduction from the ossicular chain to the vestibule (Shea 1998). Cochlear otosclerosis is not affected by these procedures, but its progression can be retarded by the oral administration of sodium fluoride (Causse et al. 1993).

Although 40 to 50% of cases appear to be sporadic, the hereditary nature of otosclerosis in other cases is well established and was recognized by Toynbee as early as 1861 (Toynbee 1861). A genetic etiology was also strongly suggested by the high concordance rate observed for monozygotic twins with otosclerosis (Fowler 1966). Most studies have concluded that inheritance of otosclerosis is autosomal dominant with reduced penetrance (Causse and Causse 1984; Gapany-Gapanavicius 1975; Larsson 1960; Morrison 1967). However, digenic inheritance of autosomal recessive genes (Bauer and Stein 1925), as well as autosomal recessive and X-linked dominant genes (Hernandez-Orozco and Courtney 1964) have been proposed. These data, as well as other epidemiologic, clinical, and molecular studies indicate *in toto* that otosclerosis is not a simple monogenic Mendelian trait, but has a multifactorial, if not multigenic, etiology and pathogenesis.

Several different lines of evidence have implicated nongenetic factors. There is a slight preponderance of females among reported cases of otosclerosis and numerous reports of hearing loss exacerbation during pregnancy, suggesting an influence of sex hormones on progression, but not necessarily prevalence, of the otosclerotic process. Other studies have addressed the possibility of a viral etiology for otosclerosis. Mumps, rubella, and measles virus antigens have all been detected in otosclerotic foci, and recent studies utilizing RT-PCR have demonstrated measles virus RNA in otosclerotic temporal bones (McKenna et al. 1996; Niedermeyer and Arnold 1995). Viral material was not detected in control temporal bone specimens in these analyses, supporting the hypothesis of a specific association of viral infection with otosclerosis, although the evidence does not establish a causal link. Finally, others have implicated immune mechanisms in otosclerosis, including autoimmunity to type II collagen (Yoo 1984). Oto-

sclerosis is likely to result from an interplay between at least some or all of these genetic, hormonal, infectious, and immunologic factors.

4.1 *A Locus Associated with an Otosclerotic Phenotype*

One important advance has been the mapping of a locus for otosclerotic hearing loss (OTS) to chromosome 15q25-q26 in a single family from India with no recorded consanguinity (Tomek et al. 1998) (Table 6.1). A somewhat higher degree of penetrance in this kindred facilitated the detection of linkage, as only three of 16 family members who inherited the OTS-linked haplotype did not have clinically detectable otosclerosis. The identification of a gene associated with otosclerotic hearing loss would provide an important molecular foundation for delineating this complex process, although mutations in *OTS* may not account for many, if not most, cases of otosclerosis.

4.2 *Osteogenesis Imperfecta (OI) and Hearing Loss*

Osteogenesis imperfecta (OI; chromosome 7) is a syndrome known to cause a stapes fixation phenotype similar to that of otosclerosis. OI is a dominant disorder caused by mutations in the $\alpha 1$ or $\alpha 2$ subunits of type I collagen, which result in abnormal bone remodeling and formation (Byers 1993). The OI phenotype is variably expressed and includes brittle or deformed bones, hyperextensible joints, and blue sclerae in addition to conductive hearing loss. An allele association study demonstrated linkage disequilibrium between otosclerosis and markers at the *COL1A1* locus encoding the $\alpha 1$ subunit of type I collagen (McKenna et al. 1998). The authors hypothesized that otosclerosis may be associated with heterozygous null alleles of *COL1A1* that are similar to those found in mild cases of OI.

These results suggest models for the etiology of otosclerosis. One model is that histologic otosclerosis is caused by a viral infection in individuals carrying heterozygous mutations in *COL1A1*, *OTS*, or other genes yet to be identified. Good candidates would be genes encoding extracellular matrix molecules, such as other collagens. The subsequent progression of otosclerosis might then be affected by hormonal factors such as those associated with pregnancy. The causal relationship between viral infection and otosclerosis may be direct or indirect, involving immune or autoimmune mechanisms that are triggered by the infection.

These first steps toward the identification of genetic loci associated with otosclerosis provide an important foundation for testing these models. Future identification of molecular genotypes at *COL1A1* and *OTS* will help clarify the roles of other causative factors. The elucidation of complex multigenic traits in other systems is just beginning to evolve, and otosclerosis should be an excellent auditory model system in which to apply those approaches.

5. Syndromic Hearing Impairment

Hearing loss may occur in association with pathologies affecting virtually any of the other organ systems, in which case it is called syndromic deafness. There are at least several hundred forms of syndromic hearing loss that are postulated to account for approximately one-third of the cases of genetic hearing loss (Gorlin et al. 1995) (Table 6.5). Deafness syndromes and their loci are often named after the clinician(s) who discovered the syndrome, such as the Waardenburg syndrome named after Petrus J. Waardenburg. Alternatively, the name of the syndrome may be based upon the phenotype, as in Branchial-Oto-Renal syndrome (BOR; Fig. 6.1 and Table 6.5). The name for a newly described deafness syndrome can be assigned by the HUGO Nomenclature Committee before the gene is mapped. This is because the new syndrome is, by definition, different from all other described deafness syndromes. Nevertheless, two clinically distinct syndromic forms of deafness may be due to allelic mutations in the same gene (i.e., allelic heterogeneity). Examples of clinically distinct syndromes caused by allelic mutations are the Marshall and Stickler syndromes, both of which can be caused by mutations in *COL11A1* (see Section 5.1). Furthermore, Waardenburg syndrome type I (MIM 193500), Waardenburg syndrome type III (OMIM 148820) and Craniofacial-Deafness-Hand syndrome (OMIM 122880) are examples of allelic mutations of *PAX3* (Asher et al. 1996).

Identification and analysis of syndromic hearing loss genes should provide insight into all types of hearing impairment, including nonsyndromic hearing loss. For example, there are alleles of genes causing syndromic hearing loss that are associated with nonsyndromic cases. Mutations of the *MYO7A* gene can cause nonsyndromic deafness *DFNA11* and *DFNB2*, as well as hearing loss with retinitis pigmentosa in Usher syndrome type IB (Liu et al. 1997b; Liu et al. 1997c; Weil et al. 1995; Weil et al. 1997). Similarly, mutations of *PDS* may cause nonsyndromic deafness *DFNB4* or Pendred's syndrome (see Section 5.2) (Everett et al. 1997; Li et al. 1998). There will likely be additional examples of allelism of syndromic with nonsyndromic hearing loss mutations as hearing loss genes continue to be identified.

Many types of syndromic hearing loss are likely to share similar pathogenetic mechanisms in the inner ear and other affected organ systems. Elucidation of the pathogenesis of auditory dysfunction may therefore be achieved by analogy to the etiopathogenesis of disease processes occurring in the other organ systems. This is especially useful given the paucity of auditory histopathologic data for the vast majority of genetic sensorineural hearing loss. For example, the well characterized basement membrane pathology observed in the progressive nephritis of Alport syndrome (sensorineural hearing loss in association with progressive nephritis) may share some pathogenetic features with the cochlea, and could facilitate our understanding of how auditory dysfunction occurs in these patients.

TABLE 6.5. Syndromic Hearing Loss Loci

Syndrome	Inheritance and Location	Locus Symbol	Gene Product	Gene Function	Auditory Phenotype	Associated Pathology	Mouse Model	Selected References
Adrenoleuko-dystrophy	XL; Xq28	<i>ALD</i>	Homology to ATP-binding transporters	Lysosomal membrane transport?	Progressive SNHL	Progressive central nervous system demyelination; blindness		Mosser et al. 1993
Albinism-deafness syndrome	XL; Xq26.3-q27.1	<i>ADFN</i>	Unknown	Unknown	Congenital SNHL	Pigmentation abnormalities		Shiloh et al. 1990
Alport syndrome	XLD; Xq22	<i>ATS/ COL4A5</i>	Collagen $\alpha 5(IV)$	Basement membrane component	Progressive SNHL (cochlear)	Progressive nephritis; lens abnormalities		Barker et al. 1990; Lemmink et al. 1997
	AR, AD; 2q35-q37	<i>COL4A3, COL4A4</i>	Collagen $\alpha 3(IV), \alpha 4(IV)$	Basement membrane component	Same as above	Same as above	<i>Col4a3</i> $-/-$ knockout	Lemmink et al. 1994; Mochizuki et al. 1994; Lemmink et al. 1997; Cogrove et al. 1998
Alström syndrome	AR; 2p13-p12	<i>ALSS</i>	Unknown	Unknown	Progressive SNHL (cochlear)	Pigmentary retinopathy; diabetes mellitus; obesity	tubby, <i>tub</i>	Kleyn et al. 1996; Noben-Trauth et al. 1996; Collin et al. 1997
Apert syndrome	Sporadic (AD); 10q26	<i>ACSI/ FGFR2</i>	Fibroblast growth factor receptor 2	Tyrosine kinase growth factor receptor	Congenital conductive HL	Premature fusion of cranial sutures, craniofacial, digital deformities; mental retardation		Wilkie et al. 1995
Aspartylglucosaminuria	AR; 4q32-q33	<i>AGU/ AGA</i>	N-aspartyl β -glucosaminidase	Lysosomal enzyme	CHL, SNHL, or MHL	Mild bone abnormalities; progressive mental retardation; coarse facies	<i>Aga</i> $-/-$ knockout	Ikonen et al. 1991; Kaartinen et al. 1996
Beta mannosidosis	AR; 4q22-q25	<i>MANBI</i>	Beta-mannosidase	Lysosomal enzyme	Mild-mod SNHL	Severe developmental delay; coarse facies		Alkhayat et al. 1998
Biotinidase deficiency	AR; 3p25	<i>BTD</i>	biotinidase	Co-factor for carboxylases	SNHL or MHL	Metabolic acidosis; dermatologic, central nervous system abnormalities		Pomponio et al. 1995

Bjornstad syndrome	AR; 2q34-q36	<i>BJS/PTD</i>	Unknown	Unknown	Congenital severe-profound SNHL	Pili torti (flat, twisted hair)	Lubianca Neto et al. 1998
Branchio-oto-renal syndrome	AD; 8q13.3	<i>BOR/EYA1</i>	Eyes-absent 1: Ortholog of drosophila "eyes-absent" gene	Unknown	CHL, SNHL, or MHL	Preauricular pits; branchial fistulas; renal abnormalities	Abdelhak et al. 1997; Johnson et al. 1998
Branchio-otic (BO) syndrome	AD; 8q13.3	<i>BOS/EYA1</i>	Eyes absent 1: Ortholog of drosophila "eyes-absent" gene	Unknown	Same as above	Preauricular pits; branchial fistulas	Vincent et al. 1997
BO syndrome with commissural lip pits	AD; 1q31	<i>BOR2</i>	Unknown	Unknown	Same as above	Preauricular sinuses; commissural lip pits	Kumar et al. 2000
Charcot-Marie-Tooth Disease, Type 1A	AD; 17p11.2	<i>CMT1A/PMP22</i>	Peripheral myelin protein-22	Structural protein of peripheral myelin	SNHL	Motor and sensory neuropathy	Lupski et al. 1991 Kovach et al. 1999
Type 1B	AD; 1q22	<i>CMT1B/MPZ</i>	Myelin protein zero	Structural protein of peripheral myelin	SNHL	Same as above	Hayasaka et al. 1993
Type 2A	AD; 1p36-p35	<i>CMT2/ CMT2A</i>	Unknown	Unknown	SNHL	Same as above	Ben Othmane et al. 1993
Type 4A	AR; 8q13-q21.1	<i>CMT4/ CMT4A</i>	Unknown	Unknown	SNHL	Same as above	Ben Othmane et al. 1993
Type 4B	AR; 11q23	<i>CMT4B</i>	Unknown	Unknown	SNHL	Same as above	Bolino et al. 1996
X-linked dominant	XLD; Xq13.1	<i>CMTX/ CX32/ GJB1</i>	Connexin 32	Gap junction protein	SNHL	Same as above	Bergoffen et al. 1993
X-linked recessive	XLR; Xp22	<i>CMTX2</i>	Unknown	Unknown	SNHL	Same as above	Ionasescu et al. 1991

TABLE 6.5. *Continued*

Syndrome	Inheritance and Location	Locus Symbol	Gene Product	Gene Function	Auditory Phenotype	Associated Pathology	Mouse Model	Selected References
Cleidocranial dysplasia	AD; 6p21	<i>CCD/CLCD/CBFAI</i>	Core binding factor, runt domain, $\alpha 1$	Osteoblast-specific transcription factor	CHL or MHL	Absent/abnormal clavicles, other skeletal malformations	cleidocranial dysplasia, <i>Ccd</i>	Mundlos et al. 1997; Sillence et al. 1987
Cockayne's syndrome, Type I/A (classic form)	AR; Chr.5	<i>CSA/CKNI</i>	WD repeat protein	RNA polymerase II transcription ?	Juvenile-onset SNHL	Defective DNA repair; growth failure; mental retardation; central nervous system deterioration; photodermatitis; skeletal anomalies		Henning et al. 1995
Type II/B (congenital form)	10q11	<i>CSB/ERCC6</i>	DNA excision repair gene	DNA excision repair	Same as above	Same as above		Mallery et al. 1998; Troelstra et al. 1992
Coffin-Lowry syndrome	XLD; Xp22.2-p22.1	<i>CLS/RSK2/RPS6KA3</i>	Ribosomal protein S6 kinase	Mitogen-activated ser/thr kinase	Mod.-severe SNHL	Mental and somatic growth retardation; skeletal anomalies		Trivier et al. 1996
Craniofacial-deafness-hand syndrome	AD; 2q35	<i>CDHS/PAX3</i>	Paired-box DNA-binding protein	Transcription factor	SNHL	Craniofacial, hand/skeletal abnormalities		Asher et al. 1996
Craniofacial dysplasia, Jackson type	AD, AR; 5p15.2-p14.1	<i>CMDJ</i>	Unknown	Unknown	Progressive MHL	Craniofacial, skeletal abnormalities; occasional facial nerve compression/palsy		Nurnberg et al. 1997
Crouzon syndrome	AD; 10q26	<i>CFDI/FGFR2</i>	Fibroblast growth factor receptor 2	Tyrosine kinase growth factor receptor	CHL	Premature fusion of cranial sutures, craniofacial deformities; small or absent ear canal (15%)		Reardon et al. 1994

Dejerine-Sottas syndrome	AD; 17p11.2	<i>DSN/HMSN3/PMP22</i>	Peripheral myelin protein-22	Structural protein of peripheral myelin	SNHL	Motor and sensory neuropathy	Ionasescu et al. 1996
DiGeorge syndrome	Sporadic, AD, AR; 22q11	<i>DGS/DGCR</i>	Contiguous gene deletion	Multiple deleted genes	CHL, SNHL, or MHL	Aberrant development of aorta, thyroid and thymic glands; craniofacial deformities	Greenberg et al. 1988
	10p14-p13	<i>DGS2</i>	Contiguous gene deletion	Multiple deleted genes		Same as above	Daw et al. 1996; Greenberg et al. 1988
Electrocutaneously, ectodermal dysplasia, and cleft lip/palate syndrome, Type I	Sporadic (AD); 7q11.2-q21.3	<i>EECI</i>	Unknown	Unknown	Variable CHL, SNHL, or MHL	Absent fingers, lacrimal puncta; cleft lip \pm palate; abnormal pigmentation of hair	Fukushima, Ohashi, and Hasegawa 1993; Qumsiyeh 1992
Type II	19p13.1-q13.1	<i>EEC2</i>	Unknown	Unknown		Same as above	O'Quinn et al. 1998
Fabry disease	XLR; Xq22	<i>GLA</i>	α -galactosidase A	Lysosomal enzyme		Cutaneous angiokeratomas; paresthesias; cataracts	Ohshima et al. 1997; Bernstein et al. 1989
FG syndrome	XLR; Xq12-q21.31	<i>FGS</i>	Unknown	Unknown	SNHL	Mental retardation; facial dysmorphism; hypotonia; imperforate anus	Briault et al. 1997
Friedreich ataxia, type I	AR; 9q13	<i>FRDA/FRDAI</i>	Frataxin	Mitochondrial protein; iron homeostasis	Mild-mod. SNHL	Central and peripheral nervous system degeneration; loss of myelinated nerve fibers	Campuzano et al. 1996
Gustavson syndrome	XL; Xq26	<i>GUST</i>	Unknown	Unknown	Severe SNHL	Mental retardation; seizures; spasticity; progressive blindness	Malmgren et al. 1993

TABLE 6.5. Continued

Syndrome	Inheritance and Location	Locus Symbol	Gene Product	Gene Function	Auditory Phenotype	Associated Pathology	Mouse Model	Selected References
Hereditary motor and sensory neuropathy, Lom type	AR; 8q24	<i>HMSNL/ NMSL</i>	Unknown	Unknown	Progressive SNHL	Peripheral nervous system demyelination and degeneration; foot and hand skeletal deformities		Kalaydjieva et al. 1996
Hunter syndrome	XLR; Xq28	<i>IDS/ MPS2</i>	Iduronate 2-sulfatase	Lysosomal enzyme	SNHL or MHL	Central nervous system degeneration; mental retardation; craniofacial dysmorphism; dysostosis		Wilson et al. 1990
Hurler syndrome	AR; 4p16.3	<i>IDUA/ MPS</i>	α -L-iduronidase	Lysosomal enzyme	CHL or MHL	Central nervous system degeneration; mental retardation; craniofacial dysmorphism; dysostosis	<i>Idua</i> $-/-$ knockout	Scott et al. 1995; Clarke et al. 1997
Hypophosphatemia (Familial hypophosphatemic rickets)	XLD; Xp22.2-p22.1	<i>HYP1/ XLH/ HPDR1/ PHEX/ PEX</i>	Similarity to metalloproteinases	unknown	Progressive SNHL; vestibular hypofunction	Vitamin-D resistant osteomalacia	Hypophosphatemia, <i>Hyp Gyro, Gy</i>	HYP consortium 1995; Strom et al. 1997
Type II	XLD, XLR; Xp11.22	<i>HYP2/ HPDR2/ CLCN5</i>	Chloride channel 5	Voltagegated chloride channel	Same as above	Same as above		Lloyd et al. 1996
Jensen syndrome	XL; Xq22	<i>MTS/ DDP/ DFN1</i>	Unknown	Unknown	Congenital SNHL	Dementia; progressive blindness; skeletal muscle wasting		Tranebjaerg et al. 1997
Jervell and Lange-Nielsen syndrome	AR; 11p15.5	<i>JLNS1/ KVLQT1/ KCNQ1</i>	alpha subunit of I(Ks)	Delayed rectifier potassium channel	Congenital prof. SNHL	Cardiac conduction abnormality; recurrent drop attacks; sudden death		Neyroud et al. 1997; Splawski et al. 1997
	21q22.1-q22.2	<i>JLNS2/ IsK/ KCNE1</i>	beta subunit of I(Ks)	Delayed rectifier potassium channel	Same as above	Same as above	<i>Isk</i> $-/-$ knockout	Vetter et al. 1996; Schulze-Bahr et al. 1997; Tyson et al. 1997

Kallmann syndrome	XL (AD) (AR); Xp22.3	<i>KAL1/HHA/KAL1G-1/ADMLX</i>	Neural cell adhesion molecule	Axonal path-finding?	Occas mild SNHL or mod-severe MHL	Hypogonadism; anosmia, agenesis of olfactory lobes	Bick et al. 1992; Legouis et al. 1991
Kniest dysplasia (metatropic dysplasia, Type II)	Sporadic, AD; 12q13.11-q13.2	<i>COL2A1</i>	Collagen $\alpha 1$ (II)	Fibrillar collagen-cartilage	CHL, SNHL, or MHL	Skeletal abnormalities; cleft palate	Garofalo et al. 1991; Vandenbergh et al. 1991; Winterpacht et al. 1993; Li et al. 1995
Krabbe disease	AR; 14q24.3-q32.1	<i>GALC/GLD/GCL</i>	Galactosylceramide beta-galactosidase	Lysosomal enzyme	Progressive SNHL	Central nervous system degeneration; progressive blindness	Sakai et al. 1996; Wenger et al. 1997
Marfan syndrome	AD; 15q21.1	<i>MFS1/MFS/FBN1</i>	Fibrillin-1	Formation of microfibrils	CHL or SNHL	Skeletal, ocular, cardiovascular anomalies	Dietz et al. 1991
	3p24.2-p25	<i>MFS2</i>	Unknown	Unknown		Skeletal, cardiovascular anomalies	Collod et al. 1994
Marshall syndrome	AD; 1p21	<i>COL11A1</i>	Collagen $\alpha 1$ (XI)	Fibrillar collagen-cartilage	Progressive SNHL	Chondrodysplasia, <i>cho</i>	Li et al. 1995; Griffith et al. 1998; Annunen et al. 1999
Mohr-Tranebjærg syndrome	XL; Xq22	<i>MTS/DDP/DFNI</i>	Unknown	Unknown	Progressive SNHL	Blindness; dystonia; mental deficiency; fractures	Jin et al. 1996
Multiple synostoses syndrome 1	AD; 17q21-q22	<i>SYNS1</i>	Unknown	Unknown	Progressive CHL	Premature joint fusions; skeletal abnormalities	Krakow et al. 1998
Neurofibromatosis, Type 2	AD; 22q12.2	<i>NF2/BANF/CAN/SCH</i>	MERLIN/Schwannomin	Tumor suppressor	Progressive SNHL; vestibular dysfunction	Schwannomas of other nerves; brain tumors; cataracts; café-au-lait spots; subcutaneous neurofibromas	Rouleau et al. 1993; Trofatter et al. 1993; Trofatter et al. 1993

TABLE 6.5. Continued

Syndrome	Inheritance and Location	Locus Symbol	Gene Product	Gene Function	Auditory Phenotype	Associated Pathology	Mouse Model	Selected References
Niemann-Pick disease, Type C	AR; 18q11-q12	<i>NPC/NPCI</i>	Similarity to transmembrane domains, cholesterol-sensing regions	Regulation of intra-cellular cholesterol trafficking	Progressive SNHL	Progressive neurologic deterioration due to sphingomyelin accumulation	Sphingomyelinosis, <i>spm</i>	Carstea et al. 1997; Loftus et al. 1997
Noonan syndrome	AD; 12q24	<i>NSI</i>	Unknown	Unknown	Progressive SNHL or MHL	Skeletal, craniofacial, heart anomalies; mild mental retardation; hematologic abnormalities; lymphangiomas, schwannomas		Jamieson et al. 1994
Norrie disease	XLR; Xp11.4	<i>ND/NDP</i>	Homology to mucins	role in neuroectodermal cell-cell interactions?	Progressive SNHL (cochlear)	Congenital or progressive blindness; mental deficiency	<i>NDP</i> -/- knock-out	Berger et al. 1992; Chen et al. 1992; Meindl et al. 1992; Berger et al. 1996
Ocular albinism with sensorineural deafness	Xp22.3	<i>OASD</i>	Unknown	Unknown	Late-onset progressive SNHL	Ocular albinism		Winship et al. 1993
Orofaciodigital syndrome, Type 1	XLD; Xp22.3-p22.2	<i>OFDI</i>	Unknown	Unknown	Occas CHL	Midfacial clefting; hyperplasia of oral cavity frenula; cleft tongue; hand anomalies; polycystic kidneys	X-linked polydactyly, <i>Xpl</i>	Sweet and Lane 1980; Feather et al. 1997
Osteogenesis imperfecta, Type I, Type II, Type III, Type IV	AD (AR); 17q21.31-q22.05	<i>OI/ COL1A1</i>	Collagen $\alpha 1(I)$	Fibrillar collagen-bone, tendon, skin	Progressive CHL or MHL	Brittle and deformed bones, hyperextensible joints; blue sclerae	Mov-13, retroviral insertion into <i>colla1</i> ; Transgenic internal deletion of <i>COL1A1</i>	Bonadio et al. 1990; Byers 1993; Pereira et al. 1993

7q22.1	<i>OI/ COL1A2</i>	Collagen $\alpha 2(I)$	Fibrillar collagen-bone, tendon, skin	Same as above	Same as above	Byers 1993
Osteopetrosis (Albers-Schönberg disease)	<i>AR; 11q12-q13</i>	Unknown	Unknown	MHL or CHL	Facial palsy; visual loss; generalized osteosclerosis	Heaney et al. 1998
Type II	<i>OPTA2</i>	Unknown	Unknown	CHL	Facial palsy; generalized osteosclerosis	Van Hul et al. 1997
Otopalatodigital syndrome, Type I	<i>OPDI</i>	Unknown	Unknown	CHL	Craniofacial, skeletal anomalies	Hoar et al. 1992
Otospondylomegal-epiphyseal dysplasia	<i>OSMED/ WZS/ COL11A2</i>	Collagen $\alpha 2(XI)$	Fibrillar collagen-cartilage	Mod-severe SNHL	Skeletal and craniofacial abnormalities; myopia	Vikkula et al. 1995; Philajamaa et al. 1998;
Paget disease	<i>PDBI</i>	Unknown	Unknown	CHL, MHL, or SNHL (cochlear); vestibular dysfunction	Progressive skull enlargement; bending of weight-bearing bones; neurologic deficits	Fotino et al. 1977
Pendred syndrome	<i>18q21-q22 AR; 7q31</i> <i>PDB2 PDS</i>	Unknown Similarity to transmembrane sulfate transporters	Unknown Chloride/iodine transporter?	Congenital SNHL (cochlear); vestibular dysfunction	Thyroid organification defect, goiter	Cody et al. 1997 Everett et al. 1997; Coyle et al. 1998; Scott et al. 1998
Pfeiffer syndrome	<i>AD; 8p11.2-p11.1</i> <i>ACSS/ FGFR1</i>	Fibroblast growth factor receptor 1	Tyrosine kinase growth factor receptor	CHL	Craniosynostosis; digit abnormalities	Muenke et al. 1994
	<i>10q26</i> <i>ACSS/ FGFR2</i>	Fibroblast growth factor receptor 2	Tyrosine kinase growth factor receptor	CHL	Same as above	Lajeunie et al. 1995; Rutland et al. 1995

TABLE 6.5. *Continued*

Syndrome	Inheritance and Location	Locus Symbol	Gene Product	Gene Function	Auditory Phenotype	Associated Pathology	Mouse Model	Selected References
	4p16.3	<i>ACSF/FGFR 3</i>	Fibroblast growth factor receptor 3	Tyrosine kinase growth factor receptor	CHL	Same as above		Bellus et al. 1996
Piebaldism	AD; 4q11-q12	<i>PBT/KIT</i>	KIT protooncogene	Mast/stem cell growth factor	Progressive SNHL	Pigmentation abnormalities; ataxia; mental retardation	Dominant white spotting, <i>W</i>	Geissler et al. 1988; Giebel and Spritz 1991; Spritz and Beighton 1998
Refsum disease	AR; 10pter-p11.2	<i>HMSN IV/PAHX/PHYH</i>	Phytanoyl-CoA hydroxylase	Peroxisomal enzyme	Progressive SNHL	Retinitis pigmentosa (retinal degeneration, blindness); cerebellar ataxia; increased plasma phytanic acid		Jansen et al. 1997; Mihalik et al. 1997
Refsum disease, infantile form	AR; 7q21-q22	<i>IRD/PEX1</i>	Peroxisome biogenesis factor 1	Peroxisomal matrix protein import	Prof SNHL	Retinitis pigmentosa; mental retardation; craniofacial dysmorphism; liver dysfunction; short stature		Reuber et al. 1997
Renal tubular acidosis with sensorineural deafness	AR; 2cen-q13	<i>dRTA/ATP6B1</i>	B1-subunit of H ⁺ -ATPase	Proton pump	Progressive SNHL	Impaired renal tubular acid secretion		Karet et al. 1999
Saethre-Chozen syndrome	AD; 7p21	<i>SCS/ACSF/TWIST</i>	TWIST	Transcription factor	Occas CHL or MHL	Premature fusion of cranial sutures; digit abnormalities	<i>Twist</i> +/- heterozygous knockout	el Ghouzzi et al. 1997; Howard et al. 1997; Bourgeois et al. 1998
	10q26	<i>SCS/ACSF/FGFR2</i>	Fibroblast growth factor receptor 2	Tyrosine kinase growth factor receptor	Occas CHL or MHL	Same as above	<i>Fgf2</i> -/- knockout	Arman et al. 1998; Paznekas et al. 1998

	4p16.3	<i>SCS/ ACS3/ FGFR3</i>	Fibroblast growth factor receptor 3	Tyrosine kinase growth factor receptor	Occurs CHL or MHL	Same as above	<i>Fgfr3</i> $-/-$ knockout	Colvin et al. 1996; Deng et al. 1996; Paznekas et al. 1998
Sialidosis	AR; 6p21.3	<i>NEU/ NEU1</i>	Sialidase/ Neuraminidase	Lysosomal enzyme	CHL or MHL	Central nervous system degeneration; vision loss; dysostosis; facial dysmorphism	SM/J line	Bonten et al. 1996; Pshezhetsky et al. 1997; Rottier et al. 1998
Smith-Magenis syndrome	Sporadic; 17p11.2	<i>SMS/ SMCR</i>	Contiguous gene deletion including MYO15	Multiple deleted genes	CHL, occas SNHL	Somatic, mental retardation; behavioral abnormalities; nonspecific combinations of anomalies		Chen et al. 1997; Smith et al. 1986
Spondyloepiphyseal dysplasia congenita	AD; 12q13.11-q13.2	<i>SEDC/ COL2A1</i>	Collagen $\alpha 1$ (II)	Fibrillar collagen cartilage	Occas. mod-severe high-freq. SNHL	Skeletal abnormalities; cleft palate; short stature		
Stickler syndrome, Type I	AD; 12q13.11-q13.2	<i>STL1/ COL2A1</i>	Collagen $\alpha 1$ (II)	Fibrillar collagen cartilage	Progressive SNHL, occas CHL	Skeletal and joint abnormalities; myopia; cataracts; craniofacial dysmorphism	<i>COL2A1</i> , <i>Col2a1</i> transgenic knockins; <i>Col2a1</i> $+/-$ heterozygous knockout	Garofalo et al. 1991; Vandenberg et al. 1991; Spranger et al. 1994; Li et al. 1995
Type II	AD; 6p21.3	<i>STL2/ COL11A2</i>	Collagen $\alpha 2$ (X1)	Fibrillar collagen cartilage	SNHL	Same as type I Stickler's syndrome, but no ocular manifestations		Vikkula et al. 1995; Sirko-Osadsa et al. 1998
Type III	AD; 1p21	<i>STL3/ COL11A1</i>	Collagen $\alpha 1$ (X1)	Fibrillar collagen cartilage	SNHL	Same as type I Stickler's syndrome	Chondrodysplasia, <i>cho</i>	Li et al. 1995; Richards et al. 1996; Annunen et al. 1999
Symphalangism, proximal	AD; 17q21-q22	<i>SYM1</i>	Unknown	Unknown	CHL	Fusion of extremity joints		Polymereopoulos et al. 1995
Tay-Sachs disease	AR; 15q23-q24	<i>TSD/ HEXA</i>	Hexosaminidase A	Lysosomal enzyme	SNHL	Progressive mental, motor retardation; seizures; blindness	<i>Hexa</i> $-/-$ knockout	Sango et al. 1995; Myerowitz 1997

TABLE 6.5. *Continued*

Syndrome	Inheritance and Location	Locus Symbol	Gene Product	Gene Function	Auditory Phenotype	Associated Pathology	Mouse Model	Selected References
Tietz syndrome	AD; 3p14.1-p12.3	<i>MITF</i>	Microphthalmia-associated transcription factor	Transcription factor	Congenital prof SNHL; normal vestibular function	Skin/hair albinism	microphthalmia, <i>mi</i>	Steingrimsen et al. 1994; Amiel et al. 1998; Smith et al. 1997
Townes-Brocks syndrome	AD; 16q12.1	<i>TBS/SALL1</i>	C2H2 zinc finger transcription factor	Transcription factor	SNHL	Deformities of external ears, anus, digits, kidneys, and heart		Kohlhase et al. 1998
Trichter Collins' syndrome	AD; 5q32-q33.1	<i>TCOF1/TCS/MFD1</i>	Nucleolar phosphoprotein	Nucleolar protein trafficking?	Variable CHL	Craniofacial anomalies; eyelid colobomas		Trichter Collins, syndrome collaborative group 1996; Wise et al. 1997
Usher syndrome, Type 1A	AR; 14q32	<i>USH1A/USH1</i>	Unknown	Unknown	Congenital severe-prof SNHL; absent vestibular function	Onset of retinitis pigmentosa (retinal degeneration, blindness) by 10yrs		Kaplan et al. 1992
Type 1B	11q13.5	<i>USH1B/MYO7A</i>	Type VIIA myosin-unconventional	Intracellular actin-based transport?	Same as above	Same as above	shaker-1, <i>sh1</i>	Gibson et al. 1995; Weil et al. 1995
Type 1C	11p15.1	<i>USH1C</i>	Unknown	Unknown	Same as above	Same as above		Keats et al. 1994
Type 1D	10q	<i>USH1D</i>	Unknown	Unknown	Same as above	Same as above		Wayne et al. 1996
Type 1E	21q21	<i>USH1E</i>	Unknown	Unknown	Same as above	Same as above		Chab et al. 1997
Type 1F	10	<i>USH1F</i>	Unknown	Unknown	Same as above	Same as above		Wayne et al. 1997

Type 2A	AR; 1q41	<i>USH2A</i>	Contains laminin-EGF and fibronectin domains	Extracellular matrix/adhesion molecule?	Congenital mod-severe SNHL; normal vestibular function	Onset of retinitis pigmentosa in late teens/early adulthood	Eudy et al. 1998
Type 2B	5q14.3-q21.3	<i>USH2B</i>	Unknown	Unknown	Same as above	Same as above	Pieke-Dahl et al. 1993; Pieke-Dahl et al. 1998
Type 3	AR; 3q21-q25	<i>USH3</i>	Unknown	Unknown	Progressive SNHL; normal or decreased vestibular function	Variable onset of retinitis pigmentosa	Sankila et al. 1995
Van Buchem disease	AR; 17q11.2	<i>VBCH</i>	Unknown	Unknown	MHL or SNHL	Skeletal hyperostosis	Van Hul et al. 1998
Velocardiofacial (Shprintzen) syndrome	AD; 22q11	<i>VCF3</i>	Frequent contiguous gene deletion	Multiple deleted genes	CHL (assoc w/otitis media), occas SNHL	Heart anomalies; facial dysmorphism; palatal cleft/dysfunction; mild mental retardation	Driscoll et al. 1992
Vohwinkel syndrome, classic form	13q12	<i>KHM/GJB2/CX26</i>	Gap junction beta-2 subunit	Gap junction subunit	SNHL	Mutilating keratoderma	Maestri et al. 1999
Vohwinkel syndrome, variant form	AD; 1q21	<i>LOR</i>	loricin	Structural component of cell envelope of epidermis	Congenital and/or progressive SNHL	Hyperkeratosis and other skin anomalies	Maestri et al. 1996
Waardenburg syndrome, Type I	AD; 2q35	<i>WS1/PAX3</i>	Paired-box DNA-binding protein	Transcription factor	Occas congenital, variable SNHL	Craniofacial dysmorphism, including dystopia canthorum; pigmentation abnormalities	Epstein, Vekemans, and Gros 1991; Baldwin et al. 1992; Tassabehji et al. 1992
Type II	AD; 3p14.1-p12.3	<i>WS2/WS2A/MITF</i>	Microphthalmia -associated transcription factor	Transcription factor	Same as WS1, SNHL may be progressive	Craniofacial dysmorphism without dystopia canthorum; pigmentation abnormalities	Steingrimsdottir et al. 1994; Tassabehji, Newton, and Read 1994

TABLE 6.5. *Continued*

Syndrome	Inheritance and Location	Locus Symbol	Gene Product	Gene Function	Auditory Phenotype	Associated Pathology	Mouse Model	Selected References
Type II, with ocular albinism	Autosomal digenic; 3p14.1-p12.3	WS2- <i>OA</i> / <i>MITF</i>	Microphthalmia-associated transcription factor	Transcription factor	Progressive SNHL	Ocular albinism		Bard, 1978; Morell et al. 1997
	11q14-q21	<i>OCA1</i> / <i>TYR</i>	Tyrosinase	Tyrosinase				
Type III (Klein-Waardenburg)	AD; AR; 2q35	WS3/ <i>PAX3</i>	Paired-box DNA-binding protein	Transcription factor	SNHL	Same as WS1, with skeletal abnormalities	spotch, <i>sp</i>	Epstein et al. 1991; Hoth et al. 1993; Zlotogora et al. 1995
Type IV (Shah-Waardenburg)	AR; 20q13.2-q13.3	WS4/ <i>EDN3</i>	Endothelin-3	Extracellular signalling peptide	SNHL	Same as WS2, with Hirschsprung disease (lack of autonomic innervation to colon)	Spotting lethal, <i>sl</i> ; <i>edn3</i> <i>-/-</i> knock-out	Baynash et al. 1994; Edery et al. 1996; Holstra et al. 1996
	AR; 13q22	WS4/ <i>EDNRB</i>	Endothelin receptor, type B	G protein-coupled receptor	SNHL	Same as above	<i>ednrb</i> <i>-/-</i> knock-out	Hosoda et al. 1994; Puffenberger et al. 1994; Attie et al. 1995
	AD; 22q13	WS4/ <i>SOX10</i>	SRY-related transcription factor	Transcription factor	SNHL	Same as above	Dominant megacolon, <i>Dmi</i>	Herbarth et al. 1998; Pingault et al. 1998; Southard-Smith et al. 1998
Wolfram syndrome	AR; 4p16.1	WFS/ <i>WFS1c</i>	Wolframin	Unknown	Progressive SNHL, HF > LF	Progressive blindness, diabetes mellitus, diabetes insipidus		Inoue et al. 1998; Strom et al. 1998
Xeroderma pigmentosum, group A	AR; 9q22.3-q31	<i>XPA</i> / <i>XPI1</i> / <i>XPAC</i>	Zinc finger domain protein	DNA excision repair	Progressive SNHL, HF > LF	Photosensitivity; cutaneous malignancies; neurologic abnormalities	<i>Xpa</i> <i>-/-</i> knock-out	Tanaka et al. 1990; de Vries et al. 1995; Nakane et al. 1995

A possible pitfall to this line of associative reasoning is that a syndrome may be caused by a contiguous gene deletion, or by linked mutations in separate genes. In this circumstance, a hearing loss gene may be altered in association with a separate gene(s) causing pathology in another organ system. This was observed in a kindred originally used to identify *DFNB1* (Kelsell et al. 1997) in which autosomal dominant palmoplantar keratoderma (PPK) was also segregating. Clinical ascertainment of that pedigree identified 3 individuals with PPK but without hearing loss, and linkage of *GJB2* was demonstrated with the hearing loss phenotype but not the PPK phenotype. This kindred illustrates how contiguous gene deletions or co-segregation of mutations have the potential to confound the linkage analysis and any conclusions regarding shared pathogenetic mechanisms between the affected organ systems.

Approximately 100 genes for syndromic hearing loss have now been mapped, and over 60 of these have been identified (Table 6.5). Some of these genes and their corresponding mutations have provided interesting and novel insights into the development and function of the auditory system. The following review will focus on six forms of syndromic hearing loss in which at least some of the causative genes have been identified, and the resulting molecular data has raised and/or answered important questions regarding the molecular basis of auditory function and hearing impairment. A discussion of all forms of syndromic hearing loss (Gorlin et al. 1995) in which the genes have been mapped or identified would require its own volume. Table 6.5 summarizes some of the essential features of approximately 100 forms of syndromic hearing loss.

It is clinically important to identify the cause of hearing loss in families where in auditory dysfunction is accompanied by other serious problems, such as heart conduction problems (Jervell and Lange-Nielsen syndrome), progressive nephritis (Alport syndrome), or progressive loss of vision (Usher syndrome). Within the congenitally hearing impaired population, the incidences of these three syndromes are estimated to be 0.25%, 1.0%, and 3 to 8%, respectively (Gorlin et al. 1995; Vernon 1959). Hearing loss may be detected before manifestation of other organ system pathologies in all three of these syndromes. For example, the hearing loss in Jervell and Lange-Nielsen syndrome may be evident before the onset of fainting attacks or detection of a cardiac arrhythmia. Life-saving anti-arrhythmic therapy may thus be initiated prophylactically (Ackerman 1998).

5.1 *Stickler Syndrome*

5.1.1 Phenotype

Stickler syndrome (OMIM 108300, 184840) is an autosomal-dominant disorder characterized by progressive sensorineural hearing loss, skeletal dysplasia, craniofacial dysmorphism, cataracts, and myopia. Marshall syndrome

(OMIM 154780) has a similar phenotype, but may be distinguished by a more severe degree of hearing loss and its unique pattern of craniofacial dysmorphism persisting into adulthood (Annunen et al. 1999; Ayme and Preus 1984; Marshall 1958) (Fig. 6.5). The sensorineural hearing loss in these disorders begins during early childhood and progresses over the ensuing decades. The hearing loss is occasionally mixed (Lucarini et al. 1987) and may be due to otitis media or its sequelae, especially in affected children.

Given the characteristic skeletal abnormalities, it is reasonable to postulate that the sensorineural hearing loss might be associated with gross morphogenetic abnormalities of the inner ear. However, there are no reports of temporal bone histopathology for these disorders, although computed tomography (CT) scans of temporal bones of 19 Stickler's syndrome patients and three Marshall syndrome patients have revealed no abnormalities (Griffith et al. 2000b; Szymko et al. 2000). Therefore, the bony anatomy of the inner ear is at least grossly normal in these disorders.



FIGURE 6.5. Frontal and side views of an individual affected with Marshall syndrome (Griffith et al. 1998). These facial features persisting into adulthood are characteristic of Marshall syndrome and include severe midfacial growth deficiency, a small upturned nose, and a prominent forehead. (Reprinted from Griffith et al., *The American Journal of Human Genetics*, Copyright 1998, with permission of The University of Chicago Press.)

Pure tone audiometry characteristically demonstrates a progressive bilateral symmetric sensorineural hearing loss predominantly affecting higher frequencies in 80% of Stickler's syndrome patients (Herrmann et al. 1975; Popkin and Polomeno 1974; Ruppert et al. 1970; Spallone 1987; Stickler and Pugh 1967; Temple 1989; Zlotogora et al. 1992). Other auditory tests indicate that the primary auditory pathology resides within the cochlea (Griffith et al. 2000b; Jacobson et al. 1990; Szymko et al. 2000).

5.1.2 Genetics of Stickler Syndrome

Stickler syndrome is genetically heterogeneous and mutations have now been identified in the genes encoding the $\alpha 1$, $\alpha 2$, and $\alpha 3$ subunits of type XI collagen: *COL11A1*, *COL11A2*, and *COL2A1* on chromosomes 6, 1, and 12, respectively (Tables 6.5, 6.6). The $\alpha 3$ (XI) subunit is translated from an alternatively spliced transcript of *COL2A1*, which encodes the $\alpha 1$ (II)

TABLE 6.6. Collagen types and their genes, chromosome map locations, and associated disorders

Type	Constituent polypeptides	Gene	Chromosome	Associated disorders
I	$\alpha 1$ (I)	<i>COL1A1</i>	17q21.31-q22.05	Ehlers-Danlos syndrome, type VII <i>Osteogenesis imperfecta</i>
	$\alpha 2$ (I)	<i>COL1A2</i>	7q22.1	Ehlers-Danlos syndrome, type VII <i>Osteogenesis imperfecta</i>
II	$\alpha 1$ (II)	<i>COL2A1</i>	12q13.11-q13.2	Achondrogenesis Hypochondrogenesis <i>Knies dysplasia</i> Spondyloepimetaphyseal dysplasia, Strudwick type <i>Spondyloepiphyseal dysplasia congenita</i> <i>Stickler syndrome, type I</i> Wagner syndrome Ehlers-Danlos syndrome, type IV Familial aortic aneurysms
III	$\alpha 1$ (III)	<i>COL3A1</i>	2q31	
IV	$\alpha 1$ (IV)	<i>COL4A1</i>	13q34	
	$\alpha 2$ (IV)	<i>COL4A2</i>	13q34	
	$\alpha 3$ (IV)	<i>COL4A3</i>	2q36-q37	<i>Alport syndrome (autosomal recessive)</i>
	$\alpha 4$ (IV)	<i>COL4A4</i>	2q36-q37	<i>Alport syndrome (autosomal recessive)</i> Benign familial hematuria
	$\alpha 5$ (IV)	<i>COL4A5</i>	Xq22	<i>Alport syndrome (X-linked)</i>
	$\alpha 6$ (IV)	<i>COL4A6</i>	Xq22	Leiomyomatosis (in association with X-linked Alport syndrome as part of a contiguous gene deletion syndrome)

TABLE 6.6. *Continued*

Type	Constituent polypeptides	Gene	Chromosome	Associated disorders
V	$\alpha 1(V)$	<i>COL5A1</i>	9q34.2-q34.3	Ehlers-Danlos syndrome, types I, II
	$\alpha 2(V)$	<i>COL5A2</i>	2q24.3-q31	Ehlers-Danlos syndrome, types I, II
VI	$\alpha 1(VI)$	<i>COL6A1</i>	21q22.3	Bethlem-type myopathy
	$\alpha 2(VI)$	<i>COL6A2</i>	21q22.3	Bethlem-type myopathy
	$\alpha 3(VI)$	<i>COL6A3</i>	2q37	Bethlem-type myopathy
VII	$\alpha 1(VII)$	<i>COL7A1</i>	3p21.3	Epidermolysis bullosa dystrophica
VIII	$\alpha 1(VIII)$	<i>COL8A1</i>	3q12-q13.1	
	$\alpha 2(VIII)$	<i>COL8A2</i>	1p34.4-p32.3	
IX	$\alpha 1(IX)$	<i>COL9A1</i>	6q13	
	$\alpha 2(IX)$	<i>COL9A2</i>	1p33-p32.2	Multiple epiphyseal dysplasia, type II
	$\alpha 3(IX)$	<i>COL9A3</i>	20q13.3	
X	$\alpha 1(X)$	<i>COL10A1</i>	6q21-q22.3	Metaphyseal chondrodysplasia, Schmid type
XI	$\alpha 1(XI)$	<i>COL11A1</i>	1p21	<i>Marshall syndrome</i>
				<i>Stickler syndrome, type III</i>
	$\alpha 2(XI)$	<i>COL11A2</i>	6p21.3	<i>Otospondylomegaepiphyseal dysplasia (OSMED)</i>
				<i>Stickler syndrome, type II</i>
	$\alpha 3(XI)^a$	<i>COL2A1</i>	12q13.11-q13.2	<i>DFNA13</i> See above for type II collagen (<i>COL2A1</i>)
XII	$\alpha 1(XII)$	<i>COL12A1</i>	6q12-q13	
XIII	$\alpha 1(XIII)$	<i>COL13A1</i>	10q22	
XIV	$\alpha 1(XIV)$	<i>COL14A1</i>	8q23	
XV	$\alpha 1(XV)$	<i>COL15A1</i>	9q21-q22	
XVI	$\alpha 1(XVI)$	<i>COL16A1</i>	1p34	
XVII	$\alpha 1(XVII)$	<i>COL17A1</i>	10q24.3	Epidermolysis bullosa, generalized atrophic benign type
XVIII	$\alpha 1(XVIII)$	<i>COL18A1</i>	21q22.3	
XIX	$\alpha 1(XIX)$	<i>COL19A1</i>	6q12-q14	

^aThe $\alpha 3(XI)$ polypeptide is a posttranslational variant of *COL2A1*.

Disorders associated with sensorineural hearing loss are italicized.

collagen polypeptide, a quantitatively major component of articular cartilage. In contrast, type XI collagen is a quantitatively minor fibrillar collagen originally isolated from articular cartilage, but subsequently shown to be present in other tissues (Bernard et al. 1988). It is formed by the association of its three constituent polypeptide subunits to form a triple helix, which is secreted into the extracellular matrix where their N- and C-terminal propeptides are cleaved (Prockop and Kivirikko 1995). The mature type XI collagen heterotrimers self-assemble into collagen fibrils whose diameter they are thought to regulate (Li et al. 1995).

The majority of reported Stickler syndrome mutations have been in *COL2A1* (Spranger et al. 1994). These dominant mutations are predicted to cause premature chain termination and are presumed to act via a haploinsufficiency mechanism. In contrast to *COL2A1*, the reported Stickler syndrome mutations in *COL11A1* and *COL11A2* are generally missense or in-frame deletion mutations that appear to act via a dominant negative mechanism (Annunen et al. 1999; Richards et al. 1996; Sirko-Osadsa et al. 1998; Vikkula et al. 1995). The Marshall syndrome phenotype appears to be specifically associated with either splice-site mutations, or genomic deletions affecting 54bp exons in the C-terminal half of *COL11A1* (Annunen et al. 1999; Griffith et al. 1998). In contrast, other mutations in *COL11A1* result in phenotypes with overlapping features of the Marshall and Stickler syndrome (Annunen et al. 1999). It is thought that these mutations of *COL11A1* and *COL11A2* exert their effects via a dominant negative mechanism in which the mutant polypeptides can initiate normal association with wild-type polypeptides via their C-terminal propeptide domains, but cannot complete proper assembly into a triple helix. The resulting mutant heterotrimers containing both wild-type and mutant polypeptides may then undergo abnormal posttranslational modification, secretion, assembly into fibrils, or degradation (Prockop and Kivirikko 1995).

The genetics of Stickler syndrome illustrate two important general principles: (1) genetic heterogeneity, in which a phenotype may be associated with mutations in one of several genes; (2) allelic heterogeneity of *COL11A1* mutations, in which more than one phenotype (the Marshall or Stickler syndromes) may be associated with mutations in a given gene. Allelism provides insights into gene structure, function and expression by providing correlations of more than one observed mutation (genotype) with a phenotype.

5.1.3 Pathogenesis of Hearing Loss in Stickler Syndrome

Biochemical and immunohistochemical analyses have demonstrated that type II collagen is expressed in the soft tissue elements of human, rodent, and avian cochleae (Khetarpal et al., 1994; Yoo and Tomoda, 1988; Ishibe, 1989; Richardson, 1987; Thalmann, 1987), as would be expected, since Stickler syndrome mutations in *COL2A1* can cause sensorineural hearing loss. Similarly, classical biochemical analyses of microdissected tissue demonstrated type XI collagen in the tectorial membrane, as well as in the basilar membrane, of the adult guinea pig (Thalmann 1993). This pattern is consistent with the pattern of *Col11a1* and *Col11a2* mRNA expression in embryonic and early postnatal mouse cochleae (McGuirt et al. 1999; Shpargel and Griffith 2000).

Fibrillar collagens are thought to be important for the observed tensile strength and compressibility of articular cartilage and other connective tissues in which they are expressed. It is possible that the Stickler and

Marshall syndrome mutations in these genes may cause sensorineural hearing loss by a direct effect on the biomechanical properties of extracellular matrices in the cochlea. Alternatively, in addition to their structural roles as extracellular matrix constituents, these mutations may regulate cochlear physiology or homeostasis via other mechanisms. Distinct roles for each of the subunits are indicated by differences in their expression patterns (Lui et al. 1995; Mayne et al. 1993; Tsumaki et al. 1996; Yoshioka et al. 1995), tissue-specific alternative splicing (Oxford et al. 1995; Yoshioka et al. 1995; Zhidkova et al. 1995), and their contributions to heterotypic fibril formation in bone and the vitreous humor of the eye (Mayne et al. 1993; Niyibizi and Eyre 1989). In addition, the mammalian discoidin domain receptors (*DDR1* and *DDR2*) with tyrosine kinase domains are activated upon receptor binding to fibrillar collagens (Shrivastava et al. 1997; Vogel et al. 1997), suggesting that extracellular collagen could directly modulate cellular responses such as growth and senescence.

5.2 *Pendred Syndrome*

5.2.1 Phenotype

Pendred syndrome (OMIM 274600) is an autosomal recessive disorder characterized by sensorineural hearing loss in association with thyroid gland enlargement. The thyroid dysfunction is biochemically characterized by defective organic binding of iodine (Fraser 1960; Sheffield et al. 1996). Serologic studies of thyroxine and thyrotropin levels may be normal, especially in patients tested early during the course of the disease (Cremers et al. 1998). The best method for detecting the thyroid organification defect is thought to be the perchlorate discharge test (Reardon et al. 1997), which reveals an abnormally high level of discharge of exogenously administered radioactive iodine from the thyroid gland in response to administration of perchlorate (Fraser 1960).

The hearing loss associated with Pendred syndrome is usually congenital and bilateral, and the severity may range from mild to profound (Kabakkaya et al. 1993). Vestibular function is variable in patients in whom it has been studied (Fraser 1965; Illum et al. 1972). A high proportion of affected individuals have radiologically detectable malformations of the inner ear (Johnsen et al. 1987). Johnsen et al. (1986) histologically studied six ears from five patients with Pendred syndrome and found bony cochlear changes consistent with the Mondini malformation in all preparations. The classic Mondini inner ear deformity includes a reduced number of turns of the cochlea, in comparison with the normal 2 1/2 turns, absence of the interscalar septum between turns of the cochlea, enlarged vestibules, abnormal semicircular canals, and enlarged vestibular aqueducts (Schuknecht 1980). A more recent study utilizing CT and MRI scanning concluded that the

Mondini cochlear deformity was a common, but not uniform, radiologic feature of Pendred syndrome (Phelps et al. 1998). In contrast, enlargement of the endolymphatic sac and duct in association with a large vestibular aqueduct was observed in all 20 patients examined by MRI (Phelps et al. 1998). Several different surgical therapies designed to alter the structure and physiology of the endolymphatic system have not been successful at preventing progression of sensorineural hearing loss associated with enlarged vestibular aqueducts (Jackler et al. 1988; Wilson et al. 1997).

5.2.2 Genetics of Pendred Syndrome

Pendred syndrome is transmitted in an autosomal-recessive pattern (Fraser 1965; Fraser 1960) and was mapped to chromosome 7q31 in a region known also to contain the nonsyndromic recessive deafness locus *DFNB4* (Baldwin et al. 1995; Coyle et al. 1996; Sheffield et al. 1996). Everett et al. (1997) identified the Pendred syndrome gene, *PDS*, by a positional cloning strategy. Northern analysis demonstrated significant expression of *PDS* in thyroid tissue. Cochlear tissue was not included in the analysis, although it was reported that PCR analyses of a human fetal cochlear cDNA library detected the presence of *PDS* sequences. *PDS* was shown to be a novel gene whose predicted protein, pendrin, shares homology to a family of transmembrane proteins that appear to be sulfate transporters (Everett et al. 1997) (Fig. 6.6). However, the expression of human pendrin in *Xenopus* oocytes is associated with transport of iodine and chloride, but not sulfate (Scott et al. 1998). Similar results were obtained in a second expression system using a baculovirus vector and Sf9 host cells (Scott et al. 1998).

Everett et al. (1997) described three different *PDS* mutations, each of which appears to act via a loss-of-function mechanism in Pendred syndrome. Two reports published together in 1998 further expanded the spectrum of known *PDS* mutations in Pendred syndrome (Fig. 6.6). Van Hauwe et al. (1998) used genomic exon sequencing to identify *PDS* mutations in fourteen of fourteen Pendred syndrome families examined from seven different countries. The results were noteworthy for the identification of two frequent missense mutations, of which one or both were present in nine of the fourteen families. Coyle et al. (1998) used SSCP to detect *PDS* mutations in 56 kindreds with features suggestive of Pendred syndrome. They identified and characterized one splice site and three missense mutations that together accounted for 74% of the detected mutations. They concluded, on the basis of analyses demonstrating common linked haplotypes, that common founders were the likely source of the common mutations (Coyle et al. 1998). The demonstration of common mutations by both groups should facilitate the molecular diagnosis of Pendred syndrome, since Pendred's syndrome is often difficult to diagnose clinically, and is probably underascertained (Reardon et al. 1997).

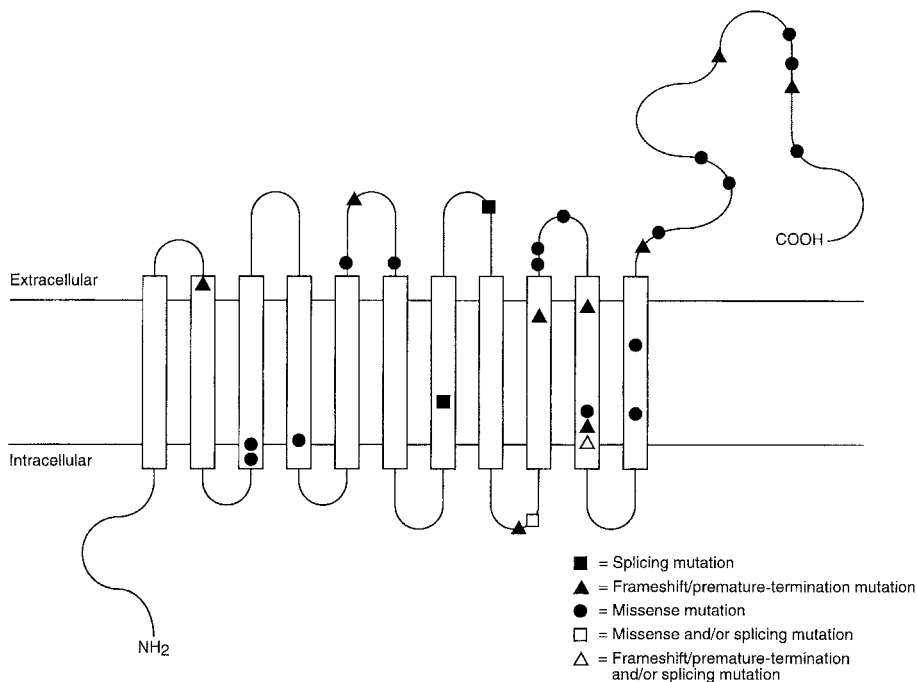


FIGURE 6.6. Postulated structure of the Pendred syndrome gene product (modified from Everett et al. 1997), and its associated mutations (Coyle et al. 1998; Cremers et al. 1998; Everett et al. 1997; Van Hauwe et al. 1998). The deduced amino acid sequence has ten predicted transmembrane domains, and is thought to transport chloride and/or iodide across cell membranes (Everett et al. 1997; Scott et al. 1998).

5.2.3 Pathogenesis of Hearing Loss in Pendred Syndrome

Establishing a direct pathogenetic link between *PDS* mutations and thyroid dysfunction is relatively simple. The demonstration that pendrin transports iodine across cell membranes suggests a direct mechanism for the observed inability to incorporate iodine into thyroid hormone in Pendred's syndrome (Scott et al. 1998).

The relationship of *PDS* to the development and function of the cochlea is less obvious. It is possible that the cochlear abnormalities are caused by thyroid dysfunction during development of the cochlea. Congenitally hypothyroid (*hyt/hyt*) mice have sensorineural hearing loss associated with abnormalities of the outer and inner hair cells (O'Malley et al. 1995). However, the cochleae of *hyt/hyt* mice are not malformed (O'Malley et al. 1995), suggesting that hypothyroidism itself does not necessarily cause cochlear malformations in mice. Therefore the inner ear malformations observed in Pendred syndrome might not be directly caused by hypothyroidism in utero. An alternative hypothesis is that pendrin plays a more

direct role in cochlear development. A direct role for pendrin in cochlear physiology and homeostasis is suggested by highly specific and discrete patterns of expression of its mRNA in the endolymphatic system, external sulcus of the cochlea, as well as the utricle and saccule (Everett et al. 1999).

5.2.4 *PDS* Mutations Cause Nonsyndromic Hearing Loss (*DFNB4*)

Several recent reports of *PDS* mutations in human subjects with nonsyndromic hearing impairment provide preliminary evidence that the observed auditory abnormalities are not a secondary effect of thyroid dysfunction. Li et al. (1998) demonstrated co-segregation of a missense mutation in *PDS* with profound, prelingual, nonsyndromic sensorineural hearing loss associated with isolated enlargement of the vestibular aqueducts in a large Indian pedigree. Usami et al. (1998) also has presented evidence that *PDS* mutations may cause nonsyndromic sensorineural hearing loss associated with enlarged vestibular aqueducts.

The *PDS* gene and its associated mutations illustrate several important principles of auditory genetics: First, both syndromic and nonsyndromic deafness may be caused by mutations in the same gene. It is therefore important to consider syndromic hearing loss genes as candidates for nonsyndromic hearing impairment, and vice versa (Fig. 6.1). Second, it is essential to thoroughly ascertain hearing loss families for syndromic manifestations, such as goiter. Reascertainment of the family originally used to map *DFNB4* revealed the presence of goiters in affected individuals, thus establishing the diagnosis of Pendred syndrome (B. Bonne-Tamir, personal communication).

5.3 *Waardenburg Syndrome*

5.3.1 Phenotype

5.3.1.1 *Waardenburg Syndrome Types 1 and 2*

Waardenburg syndrome is characterized by autosomal-dominant transmission of sensorineural hearing loss accounting for approximately 1.4 to 2% of congenital hearing loss (Fraser 1976; Partington 1964) and patchy depigmentation affecting the skin, hair, and eyes. Neural tube defects or cleft lip/palate may also be rarely observed (Farrer et al. 1992). The two major clinical subtypes of Waardenburg syndrome are distinguished by the presence (type I; WS1; OMIM 193500) or absence (type II; WS2; OMIM 193510) of dystopia canthorum, which is a lateral displacement of the inner canthi of the eyes that can be evaluated objectively using the W biometric index (Farrer et al. 1994; Newton 1989) (Fig. 6.7). Dystopia canthorum in WS1 is highly penetrant, exceeding 90% (Arias and Mota 1978). Other features of WS1 include a wide confluent eyebrow, and a high, broad nasal root (Farrer et al. 1992). Vestibular dysfunction is common but may not be symptomatic



FIGURE 6.7. Type I Waardenburg syndrome (from Asher and Friedman 1990). Characteristic WS1 features are present including dystopia canthorum, heterochromia irides, and a white forelock. (Reproduced from Asher and Friedman, *Journal of Medical Genetics* 27:617–626, Copyright 1990, with permission of the BMJ Publishing Group.)

(Marcus 1968). Iris pigmentation anomalies may include eyes of different colors, brilliant blue eyes, or two segments of different colors within a single eye. The hair, eyelashes, or eyebrows may be prematurely gray, or there may be a white forelock (Farrer et al. 1992) (Fig. 6.7). The diagnostic criteria for WS1 and WS2 have been enumerated, tabulated and reviewed (Farrer et al. 1994; Farrer et al. 1992; Liu et al. 1995; Read and Newton 1997).

There is reduced penetrance of the sensorineural hearing loss phenotype in WS1 and WS2, and within and between families the hearing loss is highly variable in severity, symmetry, onset, course, and in the observed audiometric pure-tone pattern (Fig. 6.2) (Newton 1990). Waardenburg estimated the penetrance for hearing loss at 20% (Waardenburg 1951). More recent estimates of penetrance for hearing loss are 36 to 58% for WS1, and as high as 87% in some WS2 families (Liu et al. 1995; Morell et al. 1997; Newton 1990). There are some large WS families with even higher penetrance for deafness (da-Silva 1991; Morell et al. 1992), strongly indicating the existence of modifying factors in addition to the possible effects of stochastic events (Morell et al. 1997; Pandya et al. 1996; Read and Newton 1997).

5.3.1.2 Waardenburg Syndrome Types 3 and 4

Other Waardenburg syndrome subtypes include type III Waardenburg syndrome (WS3; OMIM 148820), also known as Klein-Waardenburg syndrome. Most WS3 cases appear to be sporadic, whereas familial cases usually demonstrate autosomal-dominant inheritance. The phenotype of WS3 is composed of the WS1 phenotype in combination with hypoplastic muscles and contractures of the upper limbs. The phenotype of type IV Waardenburg syndrome (WS4; OMIM 277580), or Shah-Waardenburg syndrome, is a combination of the WS2 phenotype with Hirschsprung disease (Shah et al. 1981). The hallmark of Hirschsprung disease is colonic obstruction and dilation caused by a lack of autonomic nervous innervation to the colon.

5.3.2 Genetics of Waardenburg Syndrome

5.3.2.1 Mutations of *PAX3* Associated with WS1, WS3 and *CDHS*

WS1 was mapped to chromosome 2q based upon an initial observation of a *de novo* chromosomal inversion on 2q in a WS1 patient (Ishikiriyama et al. 1989), followed by demonstration in two families of linkage of WS1 to the placental alkaline phosphatase locus on 2q (Asher et al. 1991; Foy et al. 1990). As predicted by Asher and Friedman (1990), comparison of the map position of WS1 with the syntenic mouse region suggested a possible mouse homologue, the *Splootch* mutation (Foy et al. 1990). This hypothesis was confirmed by several groups demonstrated *PAX3* and *Pax3* mutations in WS1 patients and the *Splootch* mouse, respectively (Baldwin et al. 1992; Epstein et al. 1991; Morell et al. 1992; Tassabehji et al. 1992). *PAX3* (originally called *HuP2*) contains 10 exons encoding a transcription factor with two DNA-binding domains, a paired-domain and a paired-type homeodomain (Barber et al. 1999). Expression studies in the developing mouse demonstrate concentrated *Pax3* expression in the neural crest, as well as neural crest-derived structures (Goulding et al. 1991). The neural crest contributes to glial cells in the spiral ganglion and auditory nerve, as well as the melanocytes of the stria vascularis. Mouse *Pax3* is known to be expressed in developing limb buds (Bober et al. 1994), as predicted by the upper limb abnormalities observed in WS3.

Most, if not all, cases of WS1 map to the *PAX3* locus on chromosome 2q3. Over 50 different mutations have been described, and different families usually have different mutations (DeStefano et al. 1998). With the likely exception of missense mutations of codon 47 discussed below, most premature-termination and amino-acid-substitution mutations are predicted to cause loss-of-function and, therefore, act via haploinsufficiency. Read and Newton recently reviewed Waardenburg syndrome and favored a protein-dosage hypothesis to explain the reduced penetrance and variable expressivity of the WS1/WS3 phenotypes (Read and Newton 1997).

They postulated that effective levels of *PAX3* protein are dependent upon the *PAX3* genotype in combination with variations in the host cellular responses to *PAX3*. Any reduction of *PAX3* dosage would result in dystopia canthorum, whereas further decreases would cause, in progression, pigmentation abnormalities, limb abnormalities as seen in WS3 patients, and neural tube defects (Read and Newton 1997). This mechanism probably does not fully account for the deafness phenotype, since there is evidence that other genes, perhaps *PAX3* target genes, modify its expression (Morell et al. 1997).

Most WS3 individuals are heterozygous for *PAX3* mutations (Sheffer and Zlotogora 1992). A consanguineous marriage between two WS1 individuals heterozygous for a *PAX3* missense mutation, S84F, produced a deaf child homozygous for S84F who had dystopia canthorum, partial albinism, and severe upper-limb defects characteristic of WS3 (Zlotogora et al. 1995). Another patient with a phenotype similar to that of WS3 had an interstitial deletion, del(2)(q35–q36), that included *PAX3* and *COL4A3* (Pasteris et al. 1993). In a well studied WS3 family with four affected patients (Goodman et al. 1982; Sheffer and Zlotogora 1992), Hoth and coworkers (1993) identified an asparagine-to-histidine substitution (N47H) within the paired DNA binding domain of *PAX3*.

Craniofacial-Deafness-Hand-Syndrome (CDHS, MIM 122880) is a syndrome that resembles the WS3 phenotype. In one family, CDHS is characterized by profound sensorineural hearing loss, absence or hypoplasia of the nasal bones, hypoplastic maxilla, small and short nose with thin nares, limited wrist mobility, short palpebral fissures, ulnar deviation of the fingers, and hypertelorism (Sommer et al. 1983). Absence or hypoplasia of nasal bones was not observed in the WS3 family previously described (J. Zlotogora, personal communication). In the CDHS family, a missense mutation in codon 47 of *PAX3*, N47K, was identified (Asher et al. 1996). If the N47H mutation associated with WS3 and the N47K mutation in the CDHS family are both loss-of-function mutations, the phenotypic distinction between WS3 and CDHS, including nasal bone hypoplasia in CDHS, must be due to different alleles of modifying genes in these families. An alternative explanation is that not all mutations of *PAX3* are null alleles, and the phenotypic differences between CDHS and WS3 may be due to altered *PAX3* transcriptional properties as a consequence of the different substitutions at amino acid residue 47. N47H and N47K could differentially alter the interaction between the *PAX3* protein and a subset of its downstream target genes. This latter hypothesis may be tested when more of the *PAX3* target genes are identified.

5.3.2.2 WS2, WS2 with AROA and Tietz–Smith Syndrome

The WS2 phenotype in one kindred was mapped to chromosome 3q12–p14 by Hughes et al. (Hughes et al. 1994). This was known to be the location of the human orthologue, *MITF*, of the mouse microphthalmia gene *mi*

(Asher and Friedman 1990; Tachibana et al. 1994). Both *MITF* and *mi* encode a basic helix-loop-helix leucine zipper transcription factor. A variety of *mi* alleles exists that cause abnormalities of mast cells, teeth, osteopetrosis, or coat color (Steingrimsson et al. 1994). The *mi* protein forms dimers that can bind to the M box, a presumed cis-regulatory DNA sequence located upstream of several melanocyte-specific genes (Hemesath et al. 1994), suggesting that *mi/MITF* may be a master switch controlling melanocyte development. Finally, *PAX3* transactivates the *MITF* gene in 624 mel human melanoma cells in vitro, and WS1 mutations in the paired box or homeodomain of *PAX3* prevent binding and activation of the *MITF* promoter (Watanabe et al. 1998). These in vitro results predict that transcription of the *mi* gene would be reduced in vivo by the *Pax3* mutation in the *Splootch* mouse.

The existence of other WS2 loci is suggested by the detection of *MITF* mutations in only a minority of WS2 families studied to date. The reported *MITF* mutations appear to indicate a haploinsufficiency mechanism. All the *MITF* mutations have been associated with the typical WS2 phenotype, with two exceptions. The first is a single family with Tietz-Smith syndrome (Table 6.5), whose phenotype comprises sensorineural hearing loss and uniform pigmentation dilution. The second exception is a family with an atypical phenotype comprising WS2 features in conjunction with autosomal recessive ocular albinism (AROA; Table 6.5) (Bard 1978; Morell et al. 1997). The phenotype in this family was associated with a heterozygous *MITF* frameshift mutation in combination with a homozygous or heterozygous temperature-sensitive common polymorphism (*TYR*^{R402Q}) in the tyrosinase gene (*TYR*) that results in reduced tyrosinase catalytic activity. Heterozygotes and homozygotes for *TYR*^{R402Q} are phenotypically normal. However, compound heterozygotes between *TYR*^{R402Q} and recessive mutant alleles of *TYR* may have the AROA phenotype (Fukai et al. 1995). Morell and co-workers (1997) hypothesized that haploinsufficiency for *MITF*, the consequent down-regulation of *TYR* and the homozygosity or heterozygosity for *TYR*^{R402Q} results in the WS2 and AROA phenotype. This WS2 + AROA family is an example of digenic inheritance (Morell et al. 1997).

5.3.2.3 Molecular Genetics of WS4

Recent studies have established the molecular basis for WS4, or Shah-Waardenburg syndrome. Initial clinical observations indicated that WS4 inheritance is autosomal-recessive (Shah et al. 1981). More recent studies have shown that homozygous mutations in the endothelin-B receptor gene *EDNRB* can cause the WS4 phenotype (Attie et al. 1995; Puffenberger et al. 1994), whereas heterozygotes are normal or have Hirschsprung's disease alone (Amiel et al. 1996; Kusafuka et al. 1996). Homozygous mutations in the *EDN3* gene encoding the ligand for the endothelin-B receptor, endothelin-3, may also be associated with WS4 (Edery et al. 1996; Hofstra

et al. 1996). In addition, heterozygous *EDN3* mutations have also been demonstrated in isolated Hirschsprung disease (Bidaud et al. 1997).

WS4 may also be caused by heterozygous mutations in *SOX10* (Pingault et al. 1998), encoding the transcription factor SRY-box 10. The mouse homologue, *Sox10*, was first identified as the causative gene in the mouse mutant dominant megacolon (*dom*) (Herbarth et al. 1998; Southard-Smith et al. 1998), which results in a Hirschsprung-like phenotype in conjunction with coat color spotting. Based upon the similarity of the *dom* and WS4 phenotypes, and expression of *Sox10* in neural crest structures, Pingault et al. (Pingault et al. 1998) examined the role of *SOX10* in WS4. They found four mutations, all of which predicted to cause loss-of-function and haploinsufficiency. These findings suggest a relationship between the *EDN3/EDNRB* signaling system and *SOX10*, which has yet to be delineated. They also suggest a relationship of these genes to *PAX3* and *MITF*. It is possible that *EDN3*, *EDNRB*, or *SOX10* mutations may account for cases of WS1 or WS2 that do not have *PAX3* or *MITF* mutations. However, no such mutations have been reported to date (Read and Newton 1997), and it seems likely that other gene loci are associated with Waardenburg syndrome.

5.3.3 Pathogenesis of Hearing Loss in Waardenburg Syndrome

The genetic data have given some clues to the molecular basis of sensorineural hearing loss in Waardenburg syndrome. At least one cause of auditory dysfunction is aberrant development of those cells composing the intermediate layer of the stria vascularis that are derived from neural crest precursors. This was predicted by histopathologic studies of temporal bones from humans with Waardenburg syndrome demonstrating strial atrophy. Although it is often difficult to discern primary neuroepithelial from cochleosaccular degeneration in human temporal bone specimens (Smith et al. 1992), the differing patterns of neuroepithelial degeneration in the Waardenburg syndrome specimens were most likely secondary to strial dysfunction with loss of the endocochlear potential (Fisch 1959; Nakashima et al. 1992; Rarey and Davis 1984; Steel et al. 1987). It is also possible that the hearing loss of Waardenburg syndrome may have a digenic etiology in at least some cases (Balciuniene et al. 1998; Chen et al. 1997; Morell et al. 1997). According to this model, auditory dysfunction would be observed with strial dysfunction due to heterozygous *PAX3* or *MITF* mutations in combination with a mutant allele at another locus. These hypotheses may now be tested in animal models, such as the multiple mouse mutant models for Waardenburg syndrome that are available.

5.3.4 Animal Models of Waardenburg Syndrome

There is already at least one mouse mutant line available for each of the genes implicated in Waardenburg syndrome (Table 6.5). Several of

these mutations were critical to identification of the corresponding human genes in Waardenburg syndrome. Interestingly, auditory function analyses of *Splotch* (*Sp*+) mutant mice demonstrate that they do not have hearing impairment (Steel and Smith 1992). This may be due to the type and position of the *Splotch* mutation in *Pax3*, or to the effect of other modifying genes with alleles that functionally differ between different mouse strains (Steel and Smith 1992). A lack of significant correlation of *PAX3* genotype with the presence of hearing loss in WS1 supports the latter hypothesis (DeStefano et al. 1998). Furthermore, the influence of modifying factors is strongly indicated by the low penetrance and highly variable expressivity of the WS phenotype, and the observation that hearing loss is non-randomly distributed among families segregating WS1 (Morell et al. 1997). Moreover, Asher et al. (Asher et al. 1996) described a dissection of genetic modifiers of expression of the *Pax3*-associated phenotype in *Splotch* mice. They presented evidence that there are at least two modifying loci, one of which is probably X-linked and the other autosomal. In comparison with human families, an animal model such as *Splotch* should simplify the identification of loci modifying *PAX3* mutant phenotypes. This illustrates how characterization of genes underlying monogenic traits is establishing the foundation for identification of modifying loci in complex multigenic traits. Genetic dissection of multigenic traits is becoming an increasingly important and immediate challenge facing human geneticists because most traits and diseases are not usually monogenic, including many phenotypes of immense public health importance such as hypertension and atherosclerosis.

5.4 Usher Syndrome

5.4.1 Phenotype

Usher syndrome is an autosomal recessive disorder comprising sensorineural hearing loss and retinitis pigmentosa, a noninflammatory progressive degeneration of the retina eventually leading to blindness. Usher syndrome accounts for approximately 3 to 6% of congenital profound deafness in children, and 50% of the deaf-blind population (Vernon 1959).

Usher syndrome is clinically and genetically heterogeneous. There are three well established clinical subtypes of Usher syndrome based upon the onset of retinitis pigmentosa, the severity and onset of hearing loss, and the presence or absence of peripheral vestibular function (Smith et al. 1994). The Usher syndrome type 1 phenotype is congenital profound hearing loss with prepubertal onset of retinitis pigmentosa, and absent peripheral vestibular responses. The type 2 phenotype is congenital stable severe hearing loss with postpubertal onset of retinitis pigmentosa, and normal peripheral vestibular function. The typical audiometric pattern of type 2 is a mild low-frequency sensorineural hearing loss down-sloping to severe or

profound levels at high frequencies. The type 3 phenotype includes progressive hearing loss with variable onset of retinitis pigmentosa.

5.4.2 Genetics of Usher Syndrome

Autosomal recessive inheritance has been well established for Usher syndrome. There is significant genetic heterogeneity, with six genetically mapped loci for type 1 (*USH1A*, *USH1B*, *USH1C*, *USH1D*, *USH1E*, and *USH1F*), two loci for type 2 (*USH2A*, *USH2B*), and one locus for type 3 (*USH3*; see Fig. 6.1 and Table 6.5). Moreover, in other families the Usher syndrome phenotype is not linked to any of these known loci, indicating the existence of additional Usher syndrome loci (Pieke-Dahl et al. 1998).

5.4.2.1 Usher Syndrome Type 1B is Caused by Mutations in Myosin VIIA

Two Usher syndrome loci have now been identified (Table 6.5). The first Usher syndrome locus (*USH1B*) to be cloned was *MYO7A* on chromosome 11q13.5, encoding unconventional type VII myosin (see section 3.10) (Weil et al. 1995). Identification of *MYO7A* as the *USH1B* gene was facilitated by the recognition of a mouse deafness mutant, *shaker 1* (*sh-1*), as a possible mouse orthologue. Although *sh-1* does not have retinal degeneration, the human nonsyndromic deafness locus *DFNB2* was also known to map to the same region as *USH1B* (Guilford et al. 1994b), and therefore *sh-1* could be the mouse orthologue of *DFNB2* and *USH1B*. Gibson et al. (1995) identified mutations in a novel mouse gene encoding an unconventional myosin, *Myo7a*, associated with the *sh-1* phenotype (see Section 3.10).

Weil et al. (1995) then examined *USH1B* families for *MYO7A* mutations. Five mutations were found that were all in the motor domain and predicted to result in functional null alleles. Subsequent studies have described mutations distributed across all of the major *MYO7A* domains (Adato et al. 1997; Levy et al. 1997; Liu et al. 1998; Liu et al. 1997a; Liu et al. 1997b; Liu et al. 1997c; Weil et al. 1995; Weil et al. 1997; Weston et al. 1996) (Fig. 6.4). Liu et al. (1998) extended the known phenotypic spectrum associated with *MYO7A* by demonstrating compound heterozygous mutations in two affected siblings with an atypical Usher syndrome phenotype most closely related to that of Usher syndrome type 3.

The discovery of myosin VIIA mutations in Usher syndrome type 1B has provided new insights and raised new questions about unconventional myosins and their function within the sensory neuroepithelium of the inner ear (Gillespie et al. 1996; Hasson 1997). Unconventional myosins are known to be motor proteins that move along actin filaments in an ATP-dependent manner. Their tails are thought to associate with different macromolecular structures that are moved in relation to the actin network. Myosin VIIa has been localized to the crosslinks of adjacent stereocilia in hair cells, suggesting that myosin VIIa may be important for maintenance

of the structural integrity of hair cell bundles (Hasson et al. 1997). This postulated role for myosin VIIa is consistent with the observed histopathologic changes described for Usher syndrome (Belal 1975; Cremers and Delleman 1988; Shinkawa and Nadol 1986; van Aarem et al. 1995) and the *shaker 1* mouse (Shnerson et al. 1983).

5.4.2.2 Identification of the *USH2A* Gene

A second gene for Usher syndrome was recently described by Eudy et al. (1998). They used positional cloning methods to identify an open reading frame at the *USH2A* locus on chromosome 1q41. The corresponding cDNA was sequenced and analyzed for mutations in type 2 Usher syndrome patients who showed linkage to 1q. Heteroduplex analyses detected three different frameshift mutations that segregated with the Usher type 2 phenotype. These mutations were all predicted to cause premature chain termination and result in functional null alleles. *USH2A* expression was detected in human fetal cochlea, eye, brain and kidney, and in adult retina. The central one-third of the *USH2A* gene has ten laminin epidermal growth factor motifs arranged in tandem, which are similar to those of laminins. Laminins are extracellular proteins known to be important for cell adhesion. *USH2A* also has four tandem repeats of a fibronectin type III motif in the carboxy terminal region. Sequence analysis predicts an amino terminal signal peptide, at least two transmembrane domains, and 18 potential glycosylation sites (Eudy et al. 1998). The presence of these motifs suggests a role for the *USH2A* protein in the extracellular matrix or cell adhesion.

5.4.3 Animal Models of Usher Syndrome Type IB

There are at least seven different known alleles of *sh-1*, which are associated with *Myo7a* mutations. Gibson et al. (1995) analyzed a portion of *Myo7a* for mutations in the seven known *sh-1* alleles and found three mutations in the head region, all of which are predicted to result in null alleles. Mutations in the other four alleles have subsequently been identified, and all seven mutations are predicted to cause loss-of-function (Mburu et al. 1997). The observed pattern of neuroepithelial degeneration in *sh-1* mice and the localization of myosin VIIa to cross-links of adjacent stereocilia in hair cells implicate myosin VIIa in the maintenance of stereocilia integrity in the inner ear sensory epithelium (Hasson et al. 1997; Shnerson et al. 1983).

5.5 Alport Syndrome

5.5.1 Phenotype

The combination of hereditary sensorineural hearing loss with progressive nephritis known as Alport syndrome (OMIM 301050, 203780) accounts for

at least 1% of congenital hearing loss (Gorline et al. 1995). Alport syndrome has significant clinical and genetic heterogeneity, with most cases demonstrating X-linked dominant inheritance, while others appear to be autosomal recessive. In general, females are less affected than males in X-linked Alport cases, whereas the sexes are equally affected in autosomal forms. The age of onset and severity of disease are variable in both autosomal and X-linked forms.

The nephritis of Alport syndrome is heralded by the presence of blood in the urine. Light-microscopic evaluation demonstrates nonspecific changes in the kidneys, and ultrastructural examination reveals splitting of the glomerular basement membrane with irregular thickening or thinning. Early studies revealed absence of type IV collagen subunits in affected glomerular basement membranes, implicating it in the disease process (Jeraj et al. 1983). The nephritis of Alport syndrome may progress to renal failure and death (Flinter 1993).

The sensorineural hearing loss is bilateral, progressive, and variable in severity (Gleeson 1984). Up to 55% of males and 40% of females will develop hearing loss (Cassady 1966; Chiricosta et al. 1970). The onset is typically during the second decade of life, and high frequencies are predominantly affected (Rintelmann 1976). Speech audiometry and other audiologic tests suggest that the primary defect resides within the cochlea (Miller et al. 1970). Vestibular responses may be mildly reduced in some cases (Celis-Blaubach et al. 1974; Gleeson 1984; Miller et al. 1970). Histopathologic examinations of affected temporal bones have revealed nonspecific and inconsistent results that cannot distinguish cochlear pathology secondary to renal dysfunction from cochlear pathology occurring concomitantly with renal dysfunction (Schuknecht 1993).

The hypothesis that the cochlea and kidney may share a common pathogenic mechanism in Alport syndrome arises from the observation that they appear to share some antigenic, ultrastructural, and physiologic features. Perhaps the most notable similarity is their microvasculature and its associated basement-membrane structures that are highly specialized for the critical microfiltration processes in kidney glomeruli and the stria vascularis of the inner ear. Alternatively, the observed auditory system pathology may be secondary to kidney failure associated with increased levels of cochleotoxic factors in the blood. These factors may be endogenous metabolites, or exogenous factors commonly used to treat Alport syndrome patients, including ototoxic drugs such as aminoglycoside antibiotics and diuretics. Hemodialysis is another common therapy for the kidney failure of Alport syndrome and is associated with sensorineural hearing loss (Rizvi and Holmes 1980). It is therefore difficult to discern which cochlear pathologic changes are directly caused by the primary disease process, and which are secondary to kidney failure and its therapies.

5.5.2 Genetics of Alport Syndrome

Type IV collagen is a distinct collagen found only in basement membranes, where it is a predominant component. It is composed of three of six different polypeptide subunits ($\alpha 1$ – $\alpha 6$) which assemble into triple helices (Table 6.6). The “classical” $\alpha 1$ and $\alpha 2$ chains comprise a $[\alpha 1]_2\alpha 2(\text{IV})$ heterotrimer and a $[\alpha 1]_3(\text{IV})$ homotrimer which are ubiquitously expressed in all basement membranes. The “novel” $\alpha 3(\text{IV})$, $\alpha 4(\text{IV})$, $\alpha 5(\text{IV})$, and $\alpha 6(\text{IV})$ subunits have restricted tissue distribution and are quantitatively minor components of the basement membrane. The basement membranes of the inner ear, glomerulus, and eye are composed of two separate type IV collagen networks. One network is composed of the $\alpha 1$ and $\alpha 2$ heterotrimers, while the other network is composed of tissue-specific combinations of the other subunits. The extracellular triple helices organize via their noncollagenous amino terminal and carboxy terminal domains into a supramolecular lattice structure. Other basement membrane components such as laminin and heparin sulfate proteoglycan then assemble onto this framework.

The X-linked form of Alport syndrome is associated with mutations in the *COL4A5* gene (chromosome Xq22) encoding the $\alpha 5$ subunit of type IV collagen (Barker et al. 1990). There were over 160 different Alport syndrome mutations detected in *COL4A5* at the time this topic was reviewed in 1997 (Lemmink et al. 1997). Most mutations were either point substitutions or deletions of variable portions of the *COL4A5* gene, although larger deletions have also been reported (Lemmink et al. 1997).

In cases of parental consanguinity and equal affection status in males and females, autosomal inheritance is likely. The mapping of the type IV collagen genes *COL4A3* and *COL4A4* to chromosome 2q35–q37 suggested that they may account for autosomally inherited Alport syndrome (Mariyama et al. 1992; Turner et al. 1992). *COL4A3* and *COL4A4* are closely linked to each other in a head-to-head orientation on chromosome 2q35–q37 (Mariyama et al. 1992; Turner et al. 1992). Detection of linkage to this region and the identification of homozygous or compound heterozygous mutations in one of either of these genes established the molecular basis for autosomal forms of this disorder (Boye et al. 1998; Lemmink et al. 1994; Mochizuki et al. 1994).

5.5.3 Pathogenesis of Hearing Loss in Alport Syndrome

The identification of mutations in *COL4A3*, *COL4A4* and *COL4A5* provides a critical step toward elucidating the cause and pathogenesis of sensorineural hearing loss in this disorder. Immunohistochemical studies of type IV collagen expression of the murine cochlea demonstrated expression of *Col4a3*, *Col4a4*, and *Col4a5* in the interdental cells of the sulcus, the inner sulcus, basilar membrane, and the spiral ligament (Cosgrove et al. 1996). *Col4a5* was also heavily expressed and *Col4a3* was weakly expressed

in the stria vascularis (Cosgrove et al. 1996). Similar studies of the guinea pig demonstrated that expression of these three chains was limited to the tectorial and basilar membranes (Kalluri et al. 1998). This discrepancy in expression patterns could be due to interspecies differences, or technical variations in experimental protocol. If interspecies differences do exist, it will be even more important to determine expression patterns for the human cochlea. Accurate knowledge of these expression patterns is important, since it provides a conceptual foundation for correlating Alport syndrome genotypes with auditory system phenotypes.

5.5.4 Animal Models of Alport Syndrome

The auditory and renal phenotype of a *Col4a3* $-/-$ knock-out mouse is very similar to that observed in human patients with Alport syndrome (Cosgrove et al. 1996). ABR analyses of *Col4a3* $-/-$ mice demonstrated small increases in thresholds across all frequencies at six to eight weeks of age (Cosgrove et al. 1998). The cochlear basement membranes in *Col4a3* $-/-$ mice were either thin or absent, except in the stria vascularis, where they were significantly thickened (Cosgrove et al. 1998). This latter change was similar to the basement membrane changes observed in the glomeruli of Alport syndrome kidneys, and was associated with marked endothelial cell swelling and decreased capillary lumen size (Cosgrove et al. 1998). Furthermore, in severe cases the marginal cells of the stria exhibited pathologic changes, suggesting that perhaps the primary auditory defect may be an alteration of stria function and homeostasis. Alternatively, basement membrane changes in the basilar membrane may affect its stiffness and sound-transduction properties. However, the relevance of either model is unclear, since minimal hearing loss was observed in the mutant mice (Cosgrove et al. 1998). The reason for this lack of severe hearing loss in *Col4a3* $-/-$ mice may also underlie the reduced penetrance of sensorineural hearing loss in human patients with Alport syndrome. It is possible that other loci are modifying the auditory phenotype in *Col4a3* $-/-$ mice. This hypothesis may be addressed by breeding the mutant line on other strain backgrounds. Identification and characterization of these modifying loci, as well as other mouse strains with *Col4a4* or *Col4a5* null mutations, will shed more light on the development of sensorineural hearing loss in Alport syndrome.

5.6 Jervell and Lange-Nielsen Syndrome

5.6.1 Phenotype

Jervell and Lange-Nielsen syndrome (OMIM 220400) is an autosomal recessive disorder consisting of profound congenital sensorineural hearing loss in combination with sudden fainting attacks and a cardiac conduction abnormality characterized by a prolonged Q-T interval on an electro-

cardiogram. The frequency of this syndrome, also known as cardioauditory syndrome or surdocardiac syndrome, is about 0.25% among those with congenital profound deafness (Gorlin et al. 1995). Although rare, the importance of this syndrome lies in the high mortality rate if undetected and untreated; approximately 70% may die between early infancy and 14 years of age due to fatal cardiac arrhythmias (Gorlin et al. 1995).

The fainting spells may range from pallor and sweaty palms to loss of consciousness for up to several minutes. The episodes may begin anytime after infancy, but usually appear between three and five years of age. Therefore the hearing loss is present and detectable before the diagnosis is suggested by the fainting spells. Early detection is critical since antiarrhythmic therapy including beta-adrenergic blocking agents improves the prognosis (Ackerman 1998).

5.6.2 Jervell and Lange-Nielsen Syndrome is Genetically Heterogeneous

Autosomal recessive inheritance has been well established by frequent observations of parental consanguinity. Fraser et al. (1964) noted that heterozygotes may exhibit prolongation of the Q-T interval. Neyroud et al. (1997) analyzed four consanguineous families with Jervell and Lange-Nielsen syndrome for linkage to genes causing long-QT syndrome (without deafness), and found linkage to markers on chromosome 11p15.5 near the *KVLQTI* locus previously demonstrated to be associated with dominant long-QT (Romano-Ward) syndrome. They demonstrated a homozygous 8-bp insertion event at the site of a 7-bp deletion of wild-type DNA in the C-terminal portion of the mutant allele, causing a +1-bp frameshift that is predicted to result in premature termination. *KVLQTI* is expressed in the stria vascularis of the mouse inner ear (Neyroud et al. 1997), as would be predicted from the observed temporal bone pathology suggesting a primary defect in the stria vascularis and endolymph homeostasis (Friedmann et al. 1968; Friedmann et al. 1966).

The existence of an additional JLNS locus was indicated by the identification of two families that were not linked to *KVLQTI* (Schulze-Bahr et al. 1997). JLNS in these families was shown to be associated with mutations in *KCNE1* (chromosome 21q22.1-q22.2) (Schulze-Bahr et al. 1997), which encodes a transmembrane protein known to associate with the *KVLQTI* gene product to form the slow component of the delayed-rectifier potassium channel. *KVLQTI* encodes the alpha subunit and *KCNE1* encodes the beta subunit, IsK, of this channel. Ion-channel beta subunits are known to be ancillary proteins important for gating kinetics and stabilization of heteromultimeric channel complexes. Previous work had demonstrated expression of *KCNE1* on the endolymphatic surface of marginal cells in the stria vascularis (Sakagami et al. 1991), again consistent with a primary defect in the stria and endolymphatic homeostasis.

5.6.3 Animal Models of Jervell and Lange–Nielsen Syndrome

Vetter et al. (1996) generated and characterized a knockout mutant of the mouse gene encoding *isk*. *isk* $-/-$ mice had an auditory phenotype closely approximating that of JLNS, as well as the circling phenotype characteristic of mice with vestibular labyrinthine abnormalities. Electrophysiologic studies of the stria marginal cells and vestibular dark cells of *isk* $-/-$ mice confirmed a lack of transepithelial potassium secretion. The *isk* $-/-$ mouse strain should be useful for further investigations of endolymphatic ion homeostasis, as well as the pathophysiology associated with disruptions of this process such as that observed in JLNS (Vetter et al. 1996).

6. Conclusion

Our conceptual thinking about hearing is confined, in part, by the “deafness” genes that have and have not identified. The proper development of the auditory system and its associated electromechanical processes requires the orchestrated temporal and spatial expression of numerous different genes. Further characterization of the genes for hearing loss will provide a clearer vision of the structure and function of the auditory system in health and disease. It is hoped that these discoveries will establish a conceptual basis for the rational therapy of hearing loss and deafness.

Acknowledgments. We thank Drs. James F. Battey, Lorraine A. Everett, Penelope L. Friedman, Jeff Hung Kim, Robert J. Morell and Edward R. Wilcox for helpful comments. This work was supported by National Institute on Deafness and other Communication Disorders intramural funds Z01 DC 00035-02, Z01 DC 00039-02, Z01 DC 00040-02 and Z01 DC 00048-01 to T.B.F., and Z01 DC 00054-01 and Z01 DC 00055-01 to A.J.G.

Abbreviations

ABR	Auditory brainstem response
BAC	Bacterial artificial chromosome
bp	base pairs
BOR	Branchio-oto-renal syndrome
CHL	Conductive hearing loss
cM	CentiMorgan
CT	Computed tomography
dBHL	Decibels hearing loss
DMD	Duchenne muscular dystrophy
DPOAE	Distortion product otoacoustic emissions

EST	Expressed sequence tag
HUGO	Human Genome Organization
JLNS	Jervell and Lange-Nielsen syndrome
kb	Kilobase pairs
kDa	Kilodaltons
Lod	Logarithm of odds score
MHL	Mixed hearing loss
MIM	Mendelian Inheritance in Man
MRI	Magnetic resonance imaging
NIDCD	National Institute on Deafness and other Communication Disorders
NSRD	Nonsyndromic recessive deafness
OI	Osteogenesis imperfecta
OMIM	Online Mendelian Inheritance in Man
ORF	Open reading frame
PCR	Polymerase chain reaction
PPK	Palmoplantar keratoderma
RT-PCR	Reverse transcription-polymerase chain reaction
SNHL	Sensorineural hearing loss
SSCP	Single-strand conformation polymorphism
UTR	Untranslated region
WS	Waardenburg syndrome

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7

Hearing Loss and Mitochondrial DNA Mutations: Clinical Implications and Biological Lessons

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1. Introduction

The only known function of genes coded for by the human mitochondrial chromosome is to participate in the production of chemical energy through oxidative phosphorylation. It was thus not unexpected that mitochondrial mutations interfering with energy production could cause systemic neuromuscular disorders, which have as one of their features hearing impairment. Surprisingly, however, inherited mitochondrial mutations have also been found to be a cause of nonsyndromic and tissue-restricted hearing loss, and predispose to aminoglycoside-induced hearing loss, whereas acquired mitochondrial mutations have been proposed as one of the causes of presbycusis. This chapter will give a short review of mitochondrial genetics, outline the different mitochondrial mutations associated with inherited and ototoxic hearing loss, and discuss the clinical relevance of diagnosing these mutations. The latter part of the chapter will concentrate on the fact that the identification of these mitochondrial mutations has in most instances not yet led to an understanding of the pathophysiological steps linking the mutations to the hearing loss, nor has it allowed prediction about the clinical course and severity in any given patient. Experimental approaches to answering these questions are discussed.

2. Normal Mitochondrial Genetics

There are hundreds of mitochondria in each cell and they serve a variety of metabolic functions, the most important being the synthesis of ATP by oxidative phosphorylation. Each mitochondrion contains in its matrix 2 to 10 mitochondrial chromosomes. Each of these mitochondrial DNA (mtDNA) molecules in humans is 16,569 bp long, double-stranded, forms a closed circle, and replicates and is transcribed within the mitochondrion in ways reminiscent of its bacterial origin. The mtDNA molecule encodes 13 proteins, as well as two rRNAs and 22 tRNAs that are required for assem-

bling a functional mitochondrial protein-synthesizing system. The proteins are formed by translation of 13 mRNAs on mitochondrion-specific ribosomes, using a mitochondrion-specific genetic code. These proteins interact with approximately sixty nuclear-encoded proteins to form the five enzyme complexes required for oxidative phosphorylation. These complexes are bound to the mitochondrial inner membrane, and are involved in electron transport and ATP synthesis (reviewed in Attardi and Schatz 1988).

Mitochondrial DNA is transmitted exclusively through mothers. This leads to the expectation that a defect in a mitochondrial gene should lead to disease equally in both sexes, but can only be transmitted through the maternal line. Normally, most healthy individuals appear to have only a single mtDNA genotype (known as homoplasmy). However, in many mitochondrial disease states the mtDNA population is mixed (heteroplasmic), with both the normal and mutant genotypes present (Wallace 1992). The amount of heteroplasmy varies from tissue to tissue, and for cells within a tissue, and the severity of the symptoms does not always correlate well with the proportion of mutant mtDNAs. While for most of the multisystemic mitochondrial syndromes the homoplasmic state would presumably be lethal, mutant mtDNA homoplasmy is observed for two tissue-specific diseases, the ocular disorder Leber's hereditary optic neuroretinopathy (Howell 1994) and maternally inherited hearing loss.

3. Hearing Impairment Due to Mitochondrial DNA Mutations

Hearing loss can be due to both inherited and acquired, as well as heteroplasmic and homoplasmic, mtDNA mutations. These data have recently been reviewed (Fischel-Ghodsian, 1998a and b), and are summarized with the inclusion of the most recent data in Table 7.1.

3.1 Mitochondrial Mutations and Syndromic Hearing Loss

Systemic neuromuscular syndromes such as Kearns-Sayre syndrome, mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes (MELAS), and mitochondrial encephalomyopathy with ragged red fibers (MERRF), have hearing loss frequently as one of their clinical signs (Schon et al. 1997; Chomyn 1998; Sue et al. 1998). In these cases, the heteroplasmic mutation can be found generally at highest levels in nerves and muscle. Because of the higher energy requirements of muscle and nervous tissue, and the fact that small numbers of dysfunctional muscle and nerve cells can interrupt the function of many neighboring normal cells, mtDNA mutations in those tissues are thought to be particularly harmful. It is not unexpected

TABLE 7.1. Mitochondrial mutations and hearing impairment

Hearing impairment	Mutations identified	Inherited	Acquired	Homoplasmy	Heteroplasmy
Syndromic					
Syst. neuromuscular	del. A3243G, . . .	Rare	Usually	No	Yes
Diabetes + Deafness	A3243G-tRNA ^{Leu} (UUR)	Yes	Possible	No	Yes
	Large deletion/rearrangement	Yes	Not observed	No	Yes
	A8296G-tRNA ^{Ala}	Yes	Not known	No	Yes
	T14709C in the tRNA ^{Glu}	Yes	Not observed	No	Yes
PPK + Deafness	A7445G-non-coding	Yes	Not observed	Yes	Minimal
Nonsyndromic	A1555G-12S tRNA	Yes	Not observed	Yes	Minimal
	A7445G-non-coding	Yes	Not observed	Yes	Minimal
	Cins7472-tRNA ^{Ser} (UCN)	Yes	Not observed	Nearly	Yes
	T7511C-tRNA ^{Ser} (UCN)	Yes	Not observed	Nearly	Yes
Ototoxic	A1555G-12S tRNA	Yes	Not observed	Yes	No
	ΔT961Cn	Yes	Possible	Yes	“Multiplasmcy”
Presbycusis	“Random”	Not known	Yes	No	Yes

that generalized neuronal dysfunction is also expressed in the auditory system.

In 1992 several families with diabetes mellitus and sensorineural hearing loss were described, and surprisingly were found to have inherited the heteroplasmic A3243G mutation in the gene for *tRNA^{Leu}(UUR)* the very same mutation as is associated with the systemic MELAS syndrome (Reardon et al. 1992; van den Ouweland et al. 1992). In none of these cases were other neurological symptoms present. One family had, instead of the A3243G mutation, a heteroplasmic large deletion/insertion event (Ballinger et al. 1992), and more recently the heteroplasmic point mutations T14709C in the *tRNA^{Glu}* gene and A8296G in the *tRNA^{Lys}* gene were also found to be associated with maternally inherited diabetes and deafness (Violettes et al. 1997; Kameoka et al. 1998). This association between diabetes mellitus, hearing loss, and mtDNA mutations has been confirmed in population studies of diabetic patients (Oka et al. 1993; Alcolado et al. 1994; Kadowaki et al. 1994; Katagiri et al. 1994; Sepehrnia et al. 1995; Newkirk et al. 1997; Rigoli et al. 1997). For example, Kadowaki et al. (1994) found the A3243G mutation in 2 to 6% of diabetic patients in Japan, and in 3 out of 5 patients with diabetes and deafness. Of their 44 patients with diabetes and the A3243G mutation, 27 had hearing loss. The hearing loss is sensorineural and usually develops only after the onset of diabetes. In the non-Japanese populations that have been examined, the A3243G mutation accounts for less than 1% of the diabetic patients (Sepehrnia et al. 1995; Newkirk et al. 1997; Rigoli et al. 1997). More recently, several patients have been described with maternally inherited diabetes and deafness and also either macular dystrophy or adrenal insufficiency (Harrison et al. 1997; Nicolino et al. 1997; Souied et al. 1998). In one of these cases, the heteroplasmic A3243G mutation was found (Harrison et al. 1997), while in the other cases a heteroplasmic large deletion was identified (Nicolino et al. 1997; Souied et al. 1998).

The A7445G mutation was initially described as a nonsyndromic deafness mutation, but was subsequently found to be also associated with the skin condition palmoplantar keratoderma (PPK) in at least some of the cases (Reid et al. 1994a; Fischel-Ghodsian et al. 1995; Seviour et al. 1998). It is discussed more fully in the next section.

3.2 Mitochondrial DNA Mutations and Nonsyndromic Hearing Loss

The first mutation associated with nonsyndromic deafness was identified in an Arab-Israeli pedigree, when the striking pattern of transmission only through mothers was noted (Jaber et al. 1992; Prezant et al. 1993). Most of the deaf family members had onset of severe to profound sensorineural hearing loss during infancy, but a minority of family members had onset during childhood, or even adulthood (Braverman et al. 1996). The homo-

plasmic A1555G mutation in the mitochondrial 12S ribosomal RNA gene was identified as the pathogenic mutation (Prezant et al. 1993).

While subsequently many pedigrees and individual patients have been described with the same A1555G mutation, in all these cases the hearing loss occurred only after aminoglycoside exposure (Hutchin et al. 1993; Fischel-Ghodsian et al. 1993; Matthijs et al. 1996; Fischel-Ghodsian et al. 1997b; Pandya et al. 1997; Gardner et al. 1997). However, recently a significant number of pedigrees in Spain, with family members who went deaf with and without aminoglycosides, have been described (El-Schahawi et al. 1997; Estivill et al. 1998). In particular, the study by Estivill et al. (1998) is remarkable for two reasons, both of which indicate a higher than previously expected frequency of this mutation. First, 19 families were found to have the A1555G mutation out of the total of 70 families with sensorineural hearing loss that were included in the study. Even if the selection criteria led to a bias towards families with multiple affected individuals, and even when only the individuals without aminoglycoside exposure are considered, the frequency of familial sensorineural hearing loss due to the A1555G mutation is still unexpectedly high. Second, the fact that the mutation was identified on different haplotypes indicates that it is likely that this mutation exists in other populations as well, and may not be rare. It is also interesting to notice that the age of onset of hearing loss in the Spanish families was rarely congenital, which is different from the Arab-Israeli pedigree. Two similar families were identified recently in Italy, with 17 deaf family members who have the A1555G mutation and no exposure to aminoglycosides (Casano et al. 1998).

Another close to homoplasmic, inherited mutation leading to hearing loss is the A7445G mutation. It was first described in a family from Scotland, and confirmed in two unrelated pedigrees from New Zealand and Japan (Reid et al. 1994a; Fischel-Ghodsian et al. 1995; Seviour et al. 1998). In the New Zealand and Japanese pedigrees, the skin condition palmoplantar keratoderma also segregated in the maternal line (Seviour et al. 1998). Interestingly, the penetrance of this mutation for hearing loss in the Scottish pedigree is quite low, while in the New Zealand and Japanese pedigrees it is very high. Thus, in similarity to the Arab-Israeli pedigree, the mtDNA mutation by itself does not appear to be sufficient to cause hearing loss, but requires additional genetic or environmental factors, which seem to be rare in the Scottish pedigree and common in the New Zealand and Japanese pedigrees. The difference in penetrance in this situation appears to be due to a difference in mitochondrial haplotype. In the New Zealand pedigree, complete sequencing of the mtDNA revealed three additional sequence changes in complex I protein genes, two of which have been also labeled as secondary Leber's hereditary optic neuroretinopathy mutations (Fischel-Ghodsian et al. 1995). Since these or similar sequence changes are not present in the Scottish pedigree (Reid et al. 1994b), the mitochondrial haplotype appears to account for the differences in penetrance.

A third mtDNA mutation, a cytosine insertion at position 7472 (Cins7472) in the *tRNAser(UCN)* gene, was identified in one large Dutch family, with 27 deaf individuals (Verhoeven et al. 1999). The same mutation had been previously described in a Sicilian family with 13 members with hearing loss, seven of whom had other neurological symptoms, such as ataxia and myoclonus (Tiranti et al. 1995). In the Dutch family, only a single individual had neurological symptoms, and the hearing loss was sensorineural-progressive with onset in early adulthood. Most of the individuals over 30 years of age were deaf, indicating that the penetrance in this family is high. The mutation is heteroplasmic, although most individuals had over 90% of abnormal mitochondrial chromosomes in the tissue examined.

Recently, a large African-American pedigree with maternal inheritance of nonsyndromic hearing loss has been identified. The A1555G, A7445G, and Cins7472 mutations, as well as large deletions/rearrangements in the mtDNA were excluded, suggesting the existence of a fourth mitochondrial mutation associated with nonsyndromic hearing impairment (Friedman et al. 1999). This was confirmed with the identification of a close-to-homoplasmic mutation, T7511C, in the *tRNAser(UCN)* gene (Sue et al. 1999).

3.3 Mitochondrial DNA Mutations and Ototoxic Hearing Loss

Aminoglycoside ototoxicity is one of the most common causes of acquired deafness. Although vestibulocochlear damage is nearly universal when high drug levels are present for prolonged periods, at lower drug levels there appears to be a significant genetic component influencing susceptibility to aminoglycoside ototoxicity. The existence of families with multiple individuals with ototoxic deafness induced by aminoglycoside exposure was noticed early on. The first families with more than two members with streptomycin-induced hearing loss were described in the Japanese literature in 1957 (for review, see Higashi 1989). Prazic et al. (1964) described a family with four sisters who developed hearing loss after streptomycin injections. Tsuiki and Murai (1971) described 16 families in which two or more members had aminoglycoside ototoxicity. Konigsmark and Gorlin (1976) summarized most existing descriptions of familial aminoglycoside ototoxicity and concluded that inheritance of the predisposition is probably autosomal dominant with incomplete penetrance. However, they also noted that no male-to-male transmission has been seen, and suggested that the inheritance pattern might be multifactorial. Higashi (1989) reviewed the literature, and concluded that the most likely explanation for the maternal inheritance observed is a mitochondrial DNA defect. Hu et al. (1991) described another 36 families in China with maternally transmitted predisposition to aminoglycoside ototoxicity, and concluded also that a

mitochondrial DNA defect may be responsible. Additional evidence for a genetic basis for aminoglycoside susceptibility comes from animal studies. Macaque monkeys are resistant to dihydrostreptomycin, while the patas monkeys are highly sensitive to that drug (Stebbins et al. 1981).

Analysis of three Chinese families, in which several individuals developed deafness after the use of aminoglycosides, identified the A1555G mutation in the *12S* ribosomal RNA gene in all three of them, but not in hundreds of controls (Prezant et al. 1993). In addition, a small proportion of "sporadic" Chinese patients, without a positive family history for aminoglycoside ototoxicity, exhibit this particular mutation (Fischel-Ghodsian et al. 1993). These findings were confirmed in two Japanese families and additional Chinese sporadic cases (Hutchin et al. 1993). Subsequently, the same mutation was found in families and sporadic patients with aminoglycoside ototoxicity from Zaire, the United States, Mongolia, Spain, South Africa, and Israel (Matthijs et al. 1996; Fischel-Ghodsian et al. 1997; Pandya et al. 1997; El-Shahawi et al. 1997; Estivill et al. 1998; Gardner et al. 1997; Shohat et al. 1999). Interestingly, in one streptomycin-induced deaf individual with a strong familial history of aminoglycoside-induced hearing loss and the A1555G mutation, detailed vestibular examination revealed severe hearing loss, but completely normal vestibular function (Braverman et al. 1996).

Because the A1555G mutation in the mitochondrial *12S* rRNA gene accounts only for a minority of patients with aminoglycoside ototoxicity, it is possible that other susceptibility mutations may be found in the same gene. Mitochondrial DNA from 35 Chinese sporadic patients with aminoglycoside ototoxicity and without the A1555G mutation was analyzed for sequence variations in the *12S* rRNA gene. Three sequence changes were found, but only one of them, an absence of a thymidine at position 961 with varying numbers of cytosines inserted (Δ T961Cn), appeared likely to be a pathogenic mutation (Bacino et al. 1995). Analysis of 34 similar patients in the United States, of varying ethnic backgrounds, did not reveal this mutation, but recently an Italian family was reported in which five maternally related members became deaf after aminoglycoside treatment; they were all found to have the Δ T961Cn mutation (Casano et al. 1999). This sequence change was not found in 799 control individuals (Bacino et al. 1995).

3.4 Mitochondrial DNA Mutations and Presbycusis

Another condition associated with acquired heteroplasmic mutations and hearing loss is presbycusis. Presbycusis is the hearing loss that occurs with age in a significant proportion of individuals. Because mtDNA mutations, and the resulting loss of oxidative phosphorylation activity, seem to play an important role in the aging process (reviewed by Nagley et al. 1993), it seems likely that mtDNA mutations in the auditory system might lead to presbycusis. Recently, the spiral ganglion and membranous labyrinth from archival temporal bones of five patients with presbycusis were examined for mutations within the mitochondrially encoded cytochrome oxidase II

gene (Fischel-Ghodsian et al. 1997a). When compared with controls, the results indicated that at least a proportion of people with presbycusis have a significant load of mtDNA mutations in auditory tissue, and that there is great individual variability in both quantity and cellular location of these mutations. Similar data were obtained by Bai et al. (1997) when screening cochlear tissue from temporal bones for the presence of large deletions, although the ages of the presbycusis and control groups were not well matched. The greatest advantage of studying acquired mutations in the ear relates to the availability of temporal bone tissue banks, through which functional audiological data are available. Thus, measurable functional status, histology, immunohistochemistry of oxidative phosphorylation complexes, and mtDNA analysis can be correlated.

There are, however, two problems with the mtDNA data from patients with presbycusis. One problem relates to the fact that a sufficient number of patients has not yet been examined to draw the firm conclusion that there is indeed a clear difference between presbycusis patients and normal-hearing controls of the same age. Part of the problem is that temporal bone libraries have only the very rare patient with documented normal hearing and advanced age. But even if the data will eventually unequivocally show that presbycusis patients as a group have an increased number of mtDNA mutations, the second problem is one of causality. Are these mutations the cause or the result of the cochlear process leading to presbycusis? Two models are possible. The first model assumes that some genetic and/or environmental factors combine to cause acquired mtDNA mutations, which then lead to hearing loss. This would imply a primary causative role for mtDNA mutations. Alternatively, it is possible that the genes and environment cause presbycusis directly, and that the resulting cell death and response mechanisms lead to increased oxygen radicals and an increased rate of mtDNA mutations. In this case, the mtDNA mutations would only be a sign of presbycusis, but not a cause. To differentiate between these two models, it is necessary to use animal models in which the temporal relationship between mutations and hearing loss can be studied, as well as the response to specific interventions.

4. Clinical Relevance of Mitochondrial DNA Mutations Associated with Hearing Impairment

The major clinical relevance of mtDNA mutations to hearing loss remains the prevention of aminoglycoside-induced hearing loss. In countries where aminoglycosides are used commonly, aminoglycoside-induced ototoxicity is a major cause of hearing loss. For example, in a study that reviewed all deaf-mutes in a district of Shanghai, 21.9% had aminoglycoside-induced hearing loss, representing 167 individuals in a population of nearly half a million (Hu et al. 1991). The A1555G mutation accounted for at least 30% of these. In the United States, the A1555G mutation accounts for about 15% of all

aminoglycoside-induced deafness cases (Fischel-Ghodsian et al. 1997b). The difference in frequency may reflect the fact that aminoglycosides are generally used in the United States only for severe in-hospital infection. These patients receive significantly higher levels for more prolonged periods, and are more likely to have other medical conditions that cause or exacerbate the hearing loss. The frequency of the $\Delta T961Cn$ predisposing mutation is unknown, but it appears to be significantly lower.

Whatever the precise frequency, prevention of a major cause of aminoglycoside-induced ototoxicity is now possible. Physicians need to inquire about a family history of aminoglycoside-induced hearing loss prior to the administration of systemic aminoglycosides, as well as prior to the local administration of aminoglycosides into the cochlea as a treatment for Meniere's disease. In addition, every individual with aminoglycoside-induced hearing loss should probably be screened at least for the presence of the G1555A and $\Delta T961Cn$ mutations because presence of a mutation will allow counseling to all maternally related relatives to avoid aminoglycosides. Similarly, the study by Estivill et al. (1998) indicates that it might be reasonable to screen every individual with nonsyndromic hearing loss for the mutation, unless maternal inheritance can clearly be excluded. Because the test is easily done, and prevention of hearing loss in maternal relatives can easily be accomplished, this may be cost-effective medical practice. The tragic and avoidable hearing loss of at least forty patients in the report by Estivill et al. (1998) is a case in point.

With the exception of not administering aminoglycosides to patients with mtDNA mutations in the *12S* rRNA gene, there are no proven preventive or therapeutic interventions for mitochondrially related hearing impairments. The diagnosis of such defects is, however, useful for genetic counseling (Arnos and Oelrich, Chapter 9) and is indicated in all families with an inheritance pattern of hearing loss that is consistent with maternal transmission, and possibly in all patients who have both diabetes mellitus and adult-onset hearing loss.

5. Pathophysiology of Mitochondrial DNA Deafness Mutations

For aminoglycoside ototoxicity due to the A1555G mutation, it is interesting to notice that this mutation lies exactly in the region of the gene for which the resistance mutations in yeast and *Tetrahymena* have been described, and in which aminoglycoside binding has been documented in bacteria (Li et al. 1982; Spangler and Blackburn 1985; Gravel et al. 1987). In addition, the mutation makes the mitochondrial RNA gene in this region more similar to the bacterial ribosomal RNA gene (Prezant et al. 1993). Because aminoglycosides are concentrated within cochlear cells, and remain there for prolonged periods (Henley and Schacht 1988), it has been

proposed that susceptible individuals with the A1555G mutation have increased binding to aminoglycosides, leading to altered protein synthesis in the mitochondria (Prezant et al. 1993), and that the tissue specificity is due to the concentration of the drug in those cells. Subsequent binding experiments have proved that increased binding to the mitochondrial 12S ribosomal RNA occurs (Hamasaki and Rando 1997). However, analysis of lymphoblastoid cell lines of individuals with the A1555G mutation indicated that exposure of the cell lines to high concentrations of neomycin or paromomycin led to a decreased rate of growth in glucose medium, but no mutant proteins were detected (Guan et al. 1996). Similar results of decreased protein synthesis, but no mutant proteins were obtained using mitochondrial transfer from human skin fibroblast lines with the A1555G mutation to human ρ^0 cells (which lack mtDNA) exposed to very high levels of streptomycin (Inoue et al. 1996). This may indicate that the effect of aminoglycosides in these cell lines could be nonspecific and be different than in the cochlea, perhaps because of different transport of the antibiotic into the mitochondria.

For nonsyndromic hearing loss, at a first glance it is possible to speculate that mitochondrial mutations interfere with energy production, that the cochlea is highly dependent on sufficient energy production, and that insufficient energy production leads to degeneration of cochlear cells. However, the cochlea is not a particularly energy-dependent tissue, and in the systemic neuromuscular disorders listed in Section 3.1, the extraocular muscles appear to be the most energy-sensitive cells; hearing loss is not a prominent clinical sign in any of these mitochondrial disorders. Thus, in order to understand the pathophysiological pathways leading from the mtDNA mutations to hearing loss, two major biological questions need to be answered: Why does the same mutation cause severe hearing loss in some family members but not in others? And: Why is the ear the only organ affected?

Study of the mtDNA mutations leading to hearing loss has led to three possible precipitating factors modulating phenotypic expression, and it is likely that a combination of them also plays a significant role in the phenotypic expression of acquired mitochondrial disorders. The first such factor involves environmental agents, and aminoglycosides are the prime example as a triggering event in the case of the A1555G mutation. Other, as yet unrecognized, environmental factors could play similar, but perhaps less dramatic, roles. Diet and drugs affecting oxygen-radical formation and break-down come to mind. The second factor involves the mitochondrial haplotype, and, as noted above, the A7445G mutation provides a dramatic example of the effect of haplotype differences. The third factor involves nuclear genes. The Arab-Israeli pedigree, and some of the Spanish and Italian pedigrees, are good examples of the role of nuclear genes. For example, the entire Arab-Israeli family lives in a small Arab village in Israel, and all maternal relatives share the same mitochondrial haplotype. Bio-

chemical differences between lymphoblastoid cell lines of hearing and deaf family members with the identical mitochondrial chromosomes provide direct support for the role of nuclear factors (Guan et al. 1996). An extensive genome-wide search has led to the conclusion that this nuclear effect is unlikely to be due to a single nuclear gene (Bykhovskaya et al. 1998). Thus, the model that emerges for explaining penetrance is a threshold model, where a combination of environmental, mitochondrial, and nuclear factors can push a cell over a threshold, with dramatic clinical differences on either side of this threshold.

The second major biological question relates to tissue specificity: If a homoplasmic mutation affects oxidative phosphorylation (the only known function of the human mitochondrial chromosome and an essential process in every nucleated cell of the human body), it is unclear how the clinical defect remains confined to the cochlea, rather than affecting every tissue.

6. Experimental Approaches to Elucidate the Pathophysiology of Mitochondrial DNA Deafness Mutations

The experimental approaches to elucidate the pathophysiological pathways of the mtDNA deafness mutations are limited by the absence of a spontaneous or induced animal model for any inherited mitochondrial disorder. This is mainly due to the lack of experimental ways to manipulate the mitochondrial chromosome and transfer it into the mitochondrial cytoplasm. A collaborative study involving the Jackson Laboratories has been established to screen all hearing impaired mice for differences in their mitochondrial genomes. Pending the identification of such a spontaneous mutant, and given the lack of success with the genetic approach to identify a nuclear factor influencing the phenotype of the A1555G mutation (Bykhovskaya et al. 1998), a direct approach is proposed. It is hypothesized that degree of penetrance and tissue specificity depend on components of mitochondrial RNA processing and translation, and that cochlea-specific proteins or splice variants that interact with mutated mitochondrial RNA can be identified. Four lines of evidence implicate mitochondrial RNA processing and translation in the pathophysiological pathway between mtDNA mutation and hearing loss. Thus, components of these systems may be responsible for the tissue specificity and penetrance variability observed in patients:

(1) All the mtDNA mutations associated with nonsyndromic hearing loss involve ribosomal or transfer RNA, or in the case of the A7445G mutation, noncoding DNA. That is, none of the known mtDNA mutations associated with hearing loss causes a structural change in any of the 13 proteins

encoded by the mitochondrial genome (Table 7.1). This situation should be contrasted with other mitochondrial disorders such as Leber's hereditary optic neuroretinopathy and Leigh's syndrome, in which missense mutations are the rule (MITOMAP, 1995).

(2) Biochemical analysis of the effect of these mutations demonstrates a RNA processing defect or a decrease in translational efficiency. While the effect of these mutations has not been studied in the cochlea, analysis of lymphoblastoid cell lines has given support to the idea that the mutations affect RNA processing or translation. The A1555G mutation in the *12S* ribosomal RNA gene, which is part of the mitochondrial ribosome, causes a generalized decrease in mitochondrial protein synthesis (Guan et al. 1996). The A7445G mutation causes a processing defect of the light strand of the polycistronic mRNA, leading to a 70% reduction in *tRNA^{Ser}(UCN)* and *ND6* mRNA, which is cotranscribed with the *tRNA^{Ser}(UCN)*. The decrease in the tRNA leads to a general decrease in protein synthesis, which is exacerbated for *ND6* by the decrease of the mRNA for this protein (Guan et al. 1998). The Cins7472 mutation has not been biochemically analyzed in detail, but involves a nucleotide deletion in the T ψ C loop of the same *tRNA^{Ser}(UCN)* that the A7445G mutation affects (Tiranti et al. 1995; Verhoeven et al. 1998). The main mitochondrial mutation associated with syndromic hearing loss, the A3243G mutation in the *tRNA^{Leu}(UUR)* gene, has been proposed to interfere with transcription termination (Hess et al. 1991), although the precise mechanism of the pathogenicity of this mutation remains unclear (Moraes et al. 1992; Flierl et al. 1997).

(3) In humans and other organisms, tissue-specific isoenzymes and splice variants of genes involved in general cellular processes, also called house-keeping genes, have been described. For example, tissue-specific isoenzymes have been identified for some of the nuclear-encoded subunits of the oxidative phosphorylation complexes (Arnaudo et al. 1992), and splice variants of a mitochondrial inner-membrane phosphate carrier have been described in bovine tissues (Dolce et al. 1996).

(4) In humans and other organisms, different processing of mitochondrial RNA and protein, leading to tissue specific defects or functions, have been described. For example, in *Drosophila melanogaster*, the mitochondrial large ribosomal RNA can, in germ cell precursor cells, also be processed for export into the cytoplasm, where it induces pole cell formation in embryos, a key event in the determination of the germ line (Kobayashi et al. 1993). A human example is a case of a 22-year-old patient who died from respiratory failure due to a mitochondrial myopathy. Analysis of tissues from the patient showed that the causative mutation in the mitochondrial *tRNA^{Leu}(UUR)* gene resulted in a RNA processing defect in skeletal muscle, but not in fibroblasts (Bindoff et al. 1993). Also, in humans it has been shown that a normally occurring RNA processing intermediate of the heavy-strand polycistronic RNA is more prevalent in lymphocytes and liver than in other tissues (Nardelli et al. 1994).

Based on these four lines of evidence, it is proposed that cochlea-specific subunits of mitochondrial ribosomes or RNA processing proteins interact abnormally with the mitochondrial defect, leading to insufficient oxidative phosphorylation, or loss of a secondary function in the cochlea. Also, variation in the coding sequence for some of these proteins may explain the penetrance differences observed among patients. Mitochondrial RNA processing and translation is a complex process, which occurs exclusively in the matrix of the mitochondria. It requires nuclear-encoded proteins for every step, and has characteristics reminiscent of the bacterial origin of the mitochondrial genome. Estimates of the number of proteins involved range from 200 to 300, and only a small number of these proteins have been identified. Additional proteins can be identified by a variety of methods. For example, the mitochondrial ribosomal *S12* gene was identified by cybercloning (Johnson et al. 1998), and this protein has been used as a bait in the yeast dihybrid system to identify a second protein that is an excellent candidate for being another mitochondrial ribosomal protein. All the new proteins identified through such methods are tested for isoforms and cochlea-specific splice variants, using cochlea-specific cDNA libraries (Giersch and Morton, Chapter 3). A more direct approach to the identification of some of these proteins is through isolation of mitochondrial ribosomes from cochlea and another tissue, resolution by 2D gel electrophoresis, and microsequencing of proteins with different electrophoretic characteristics from individual spots. The bovine model system has been established for this purpose (Matthews et al. 1982; O'Brien and Denslow, 1996), and identity correspondence to the human mitoribosomal system has been established by 2D PAGE co-electrophoretic separations of human and bovine mitoribosomal proteins (Matthews et al. 1982).

7. Summary

In conclusion, mitochondria-related hearing loss can be caused by a variety of mutations, and can present in a variety of clinical forms with different degrees of severity. These mutations are not uncommon and, owing to the susceptibility of individuals with the A1555G and Δ T961Cn mutations and their maternal relatives to aminoglycosides, are important to diagnose. Despite the fact that these mostly homoplasmic mitochondrial mutations represent the simplest model of a mitochondrial DNA disease, it remains unclear how mtDNA mutations lead to the clinically crucial features of penetrance and tissue specificity. Within the same family, some individuals with the mutation can have profound hearing loss, while others have completely normal hearing, and only the hearing is affected although all tissues have the mutation and are dependent on mitochondrial ATP production. Experimental approaches using spontaneous mouse models of mitochondrial hearing impairment, or direct investigation of the most likely biochemical

pathways involved, may help not only in elucidating the pathophysiology between mtDNA mutations and hearing loss, but may also provide a paradigm for mitochondrial diseases in general.

Acknowledgments. The author is supported by NIH/NIDCD grants RO1DC01402, RO1DC02273, RO1DC03395, and RO1DC04092.

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8

Mice as Models for Human Hereditary Deafness

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1. Introduction

Investigating the biological basis of genetic hearing impairment in humans is very difficult. Temporal bone specimens can be analysed and have provided vital clues to aid gene identification in the past (Robertson et al. 1998), but in most cases these specimens come from elderly people who have end-stage cochlear pathology. Thus, such tissue may not be helpful in determining the cause of deafness early in life. A very common, if not universal, result of cochlear dysfunction is hair cell degeneration, which also leads to dedifferentiation of supporting cells, and this gives few clues to the cause of deafness. Yet, there is little evidence for the degeneration of hair cells being the cause of cochlear dysfunction, rather than the result, in genetic deafness. Some electrophysiology can be carried out in humans, and this may distinguish conductive from sensorineural deafness, and cochlear from central auditory system pathology, but detailed physiological analysis of the function of the ear, such as endocochlear potential measurements and single hair cell recordings, cannot be undertaken in humans. Developmental studies are also not possible in humans, but it is only by studying the earliest stages of dysfunction that the reasons for deafness can be uncovered.

Animal models do not have these disadvantages because they can be studied at any stage of development, and functional studies can be carried out on anaesthetised animals or isolated preparations from the ear. Also, experimental manipulations, such as transgenic or gene knockout approaches, are feasible. Furthermore, the early stages of positional cloning of deafness genes (Mueller, Van Camp, and Lench, Chapter 4) is facilitated by the ability to generate very large numbers of animals all known to be carrying the same deafness mutation. This allows much more accurate localization in mapping the mutation than could be achieved in humans with their small family sizes and heterogeneity. Mice are the obvious choice of model because of the profusion of deaf mutants already known, the rapid generation time, and the use of this species as the major mammalian model in genome analysis.

Deaf mouse mutants are therefore very helpful in studying genetic deafness, but are they good models for human hereditary deafness? The answer is, with very few exceptions, yes. The cochlea of the mouse has a virtually identical organization to that of humans, with differences, such as differences in size, being trivial. Also, the cochleas of deaf mouse mutants and profoundly hearing impaired humans show very similar types of pathology (as far as can be determined, given the limitations in studying humans). Syndromic deafness occurs in both species, and the range of associated defects in the two species is very similar, suggesting that the biological mechanisms underlying the disease are the same. A number of mouse mutants with mutations in orthologous (equivalent) genes to those known to be involved in deafness in humans have been studied, and there is generally a good correlation between the phenotypes in the two species.

There are a few exceptions to the general rule that the mouse models accurately reflect the pathology seen in humans with mutations in orthologous genes. In some cases, it is likely that genetic background differences between mice and humans can account for the differences. For example, mice with a single *Pax3* mutation are not deaf, while humans with a mutation in the same gene have Waardenburg syndrome, which includes occasional deafness (Steel and Smith 1992; Tassabehji et al. 1992). However, at least part of the phenotype of Waardenburg syndrome, the widely spaced eyes, can be seen in mice with a *Pax3* mutation on a different genetic background (Asher et al. 1996).

The mouse and human genomes show many similarities. Large stretches of chromosomes are conserved between mouse and human, with orthologous genes arranged in the same order, a phenomenon known as conserved synteny. It appears that an ancestral mammalian genome has been cut up and rearranged during the course of evolutionary divergence of the two species, with only a few tens or hundreds of cuts required to explain the present-day organization of genes. This is an extremely useful feature for the molecular geneticist. It means that genes within regions of conserved synteny can be studied in one species and the information used to predict the genes and their order in the other species. Thus, if a deafness mutation is located between two known genes in the mouse, a human deafness gene can be predicted to occur between the two human versions of the known genes. This lining up of the two genomes has been invaluable for suggesting candidate deaf mouse mutants as models for particular forms of human deafness. An example was the proposal that the *shaker1* mutation on mouse chromosome 7 might involve the same gene as in Usher syndrome type 1B on human chromosome 11q because both were linked to another gene, *Omp* (Evans et al. 1993). Positional cloning of the *Myo7a* gene involved in deafness in *shaker1* mutants led rapidly to the finding of mutations in the human version of the gene, *MYO7A*, in humans with Usher syndrome, emphasizing the value of identifying deafness genes in the mouse (Gibson et al. 1995; Weil et al. 1995). Such comparative analyses of mouse and

human genomes can also give clues to genes in the region, which can then be used for finer mapping, or for investigation as candidate genes for involvement in the deafness.

Because the sequence of the human genome, and some indication of the location of coding sequences within it, is likely to be available very soon, whereas the complete mouse sequence will be at least a couple of years later, comparisons of human and mouse sequence will be of particular importance to mouse geneticists over the next few years. There are several Web sites available that provide valuable information about specific aspects of genetic deafness, including comparisons of human and mouse deafness loci (Zheng et al. 2000) and expression of genes in the ear (Bussoli et al. 2000; Morton 2000). Johnson and colleagues also have a useful review of mouse mutants with ear defects (Johnson et al. 2000). The tables of deaf mutants presented in this chapter will be updated periodically and be available at a new Web site (Steel 2000).

There are now many different mouse mutants described in the literature with auditory-system defects of one sort or another, and more that seem to be good candidates for involvement in hearing even though they have not yet been investigated thoroughly. In this chapter, the main types of defects seen in mouse models, and how these models relate to human deafness, are discussed. Further details of mouse mutants with specific auditory-system defects reported in the literature, together with key references, are provided in the tables.

2. Classifying Defects

It can be argued that, as each mouse mutant involves a mutation in a different gene, and hence represents a unique interference in a specific molecule, there is little point in grouping mutants together according to gross pathology. However, molecules do not act in isolation, but in the context of cellular and tissue systems. Thus, mutations may affect different molecules, but still affect the same functional or developmental system. It will be important to understand these whole systems, not only to give a complete understanding of the molecular basis of normal auditory function and development for academic interest, but also because the prospects for treatment will be much improved if the whole system is understood. Because human genetic deafness is highly heterogeneous (many different genes are involved, any one of which can underlie the deafness in an individual), it is likely that the investment needed to develop any treatments will depend upon generic approaches, which deal with the whole system rather than a single step. For example, treatments might be developed to boost the proposed recycling of potassium within the cochlear duct, irrespective of which component of the cycle is the weak link. Another example is stimulation of hair cell regeneration irrespective of the cause of the

degeneration (as long as hair cell degeneration, rather than hair cell dysfunction, is the cause of the hearing loss, which is not necessarily the case in genetic deafness).

2.1 Middle Ear and Pinna Defects

In humans with syndromic hearing impairment, pinna defects are sometimes associated with middle ear defects, so it seems useful to consider these two parts together. The outer and middle ear has a complex developmental origin, with contributions from all three germ layers (endoderm, ectoderm, and mesoderm) and from the first and second branchial arches. A groove in the surface ectoderm extends inwards to meet an extension of the pharyngeal endoderm, and the two pockets meet to form the two cell layers of the tympanic membrane. The three middle ear ossicles (malleus, incus and stapes) form from condensation of mesenchymal cells within the pharyngeal pouch, which eventually loses its excess mesenchyme to form an air-filled recess, the middle ear, linked to the pharynx by the remains of the endodermal extension, the Eustachian tube. Neural crest cells migrate to fill the branchial arches in early development, and the first branchial arch contributes to the pinna, incus, malleus, and tympanic membrane, while the second arch contributes to the pinna, incus, malleus, and stapes. This complexity means that it is not surprising that many genes involved in craniofacial development are important for development of the outer and middle ear, leading to specific anomalies when the gene is interrupted by knock-out techniques. These anomalies are wide-ranging in effect, including minor differences such as small pinnae, smaller oval windows or minor malformation of ossicles, through duplication of ossicle primordia or incomplete tympanic rings, to major malformation of the entire area. Table 8.1 gives details of many of the reported pinna and middle ear defects in mutants, and Figure 8.1 (Fekete 1999) illustrates the location of the defects in several of the mutants. It is noteworthy that many of the knock-out mutants included in the table have such severe craniofacial defects that they die at an early stage, often just after birth. So far, only two appear to represent mutations in genes involved in human deafness, *Eya1* and *Pou3f4*. *Eya1* is the mouse version of the gene underlying Branchio-Oto-Renal syndrome (BOR), which shows variable middle ear malformation and mixed hearing impairment (Abdelhak et al. 1997). *Pou3f4* is the mouse orthologue of the gene involved in X-linked deafness with gusher, in which middle ear surgery to release an apparently fixed stapes results in a gush of fluid from the oval window, a consequence of an inner ear malformation that prevents the separation of inner ear perilymph from cerebrospinal fluid (de Kok et al. 1995). The mouse knockout shows some similar inner ear malformations (Phippard et al. 1999), and has also been found to have reduced endocochlear potentials (discussed later). Interestingly, deletion analysis in humans reveals a regulatory region far upstream of the coding region of

TABLE 8.1. Mouse mutants with middle ear or pinna defects

Mutant name	Symbol	Gene	Chromosome	Inheritance	Origin	Key references
Otocephaly	<i>oto</i>	NK	1	R, E	R	1, 2
Eyes absent 1 homologue KO	<i>Eya1</i>	<i>Eya1</i>	1 (10.4)	SD	T	3, 4
<i>Gli2</i> KO	<i>Gli2</i>	<i>Gli2</i>	1 (63)	R	T	5
<i>Prx2</i> KO	<i>Prx2, S8</i>	<i>Prx2</i>	2 (19)	R, E	T	6
First arch malformation	<i>far</i>	NK	2 (40)	SD	S	7, 8
Distal-less homeobox 1 KO	<i>Dlx1</i>	<i>Dlx1</i>	2 (44)	R, E	T	9
Distal-less homeobox 2 KO	<i>Dlx2</i>	<i>Dlx2</i>	2 (44)	R, E	T	9
Flaky tail	<i>ft</i>	NK	3 (41.4)	R	S	12
Droopy ear	<i>de</i>	NK	3 (48.8)	R	S	13
Dominant reduced ear	<i>Dre</i>	NK	4	SD	R	12
Perlecan KO	<i>Hspg2, Plc</i>	<i>Hspg2</i>	4 (71.4)	R	T	14
Homeobox, msh- like KO	<i>Msx1, Hox7</i>	<i>Msx1</i>	5 (21)	R	T	15
<i>Dlx5</i> KO	<i>Dlx5</i>	<i>Dlx5</i>	6 (2)	R	T	16, 17
Homeobox A1 KO	<i>Hoxa1</i>	<i>Hoxa1</i>	6 (26.3)	R, E	T	18–22
Homeobox A2 KO	<i>Hoxa2</i>	<i>Hoxa2</i>	6 (26.3)	R	T	23–26
Head bobber	<i>hb</i>	NK	7 (65)	R	I	27
Short ear	<i>se, Bmp5</i>	<i>Bmp5</i>	9 (42)	R	S, R	28, 29
Apoptotic protease activating factor 1 KO	<i>Apaf1</i>	<i>Apaf1</i>	10	R	T	30
Hemifacial microsomia- associated locus	<i>Hfjm</i>	NK	10	SD	I	31
Homeobox B1 KO	<i>Hoxb1</i>	<i>Hoxb1</i>	11 (56)	R, E	T	32–35
Homeobox B2 KO	<i>Hoxb2</i>	<i>Hoxb2</i>	11 (56)	R	T	36
Collagen type 1 α 1 KO, Mov-13	<i>Col1</i> <i>Mov-13</i>	<i>Col1</i>	11 (56)	D	T, I	37–39
Retinoic acid receptor α KO	<i>Rara</i>	<i>Rara</i>	11 (57.8)	R, E	T	40–42
<i>Pax9</i> KO	<i>Pax9</i>	<i>Pax9</i>	12 (26)	R	T	43
Goosecoid KO	<i>gsc</i>	<i>gsc</i>	12 (52)	R	T	44–46
Endothelin 1	<i>Edn1, Et1</i>	<i>Edn1</i>	13 (26)	R	T	47
Homeobox, msh-like 2 KO	<i>Msx2, Hox8</i>	<i>Msx2</i>	13 (32)	D	T	48
Cartilage link protein 1 KO	<i>Crtl1</i>	<i>Crtl1</i>	13 (44)	R	T	49
Retinoic acid receptor β KO	<i>Rarb</i>	<i>Rarb</i>	14	R, E	T	40–42
Orthodenticle homologue-2 KO	<i>Otx2</i>	<i>Otx2</i>	14 (19)	SD, E	T	50–52
Hairy ears	<i>Eh</i>	NK	15	SD	R	12

TABLE 8.1. *Continued*

Mutant name	Symbol	Gene	Chromosome	Inheritance	Origin	Key references
Retinoic acid receptor γ KO	<i>Rarg</i>	<i>Rarg</i>	15 (57.4)	R, E	T	42
Eye-ear reduction	<i>Ie</i>	NK	X	SD	R	12
Sex-linked fidget, <i>Pou3f4</i> KO	<i>slf, Brn4 Pou3f4</i>	<i>Pou3f4</i>	X (48.4)	SD	T, R	53–55
Low set ears	<i>Lse</i>	NK	NK	SD	S	56
Micropinna-microphthalmia	<i>Mp</i>	NK	NK	SD	R	12
Endothelin receptor Type A KO	<i>Ednra</i>	<i>Ednra</i>	NK	R	T	57
Endothelin converting enzyme-1 KO	<i>Ece1</i>	<i>Ece1</i>	NK	R	T	58
<i>Prx1</i> KO	<i>Prx1, Mhox</i>	<i>Prx1</i>	NK	R, E	T	6, 59
<i>p73</i> KO	<i>p73</i>	<i>p73</i>	NK	R	T	60
Small ear	<i>Sme</i>	NK	NK	D	R	12

The table includes the major genes or loci known to be involved in deafness and/or balance defects. Only the key references describing the ear phenotype and the initial identification of the gene are included, so the list is not intended to be comprehensive. The chromosomal localization is given where this is known, with the distance in cM from the centromere given in parentheses. KO, Knockout. NK, Not known. Under Inheritance column: R, Recessive; D, Dominant; SD, Semidominant; E, Epistatic; M, Maternal effect. Under Origin column: R, Radiation-induced; S, Spontaneous; T, Transgenics and knock-outs; C, Chemical mutagenesis; I, Transgenic Insertional mutations.

References: 1, Juriloff et al. 1985; 2, Zoltewitz et al. 1999; 3, Johnson et al. 1999; 4, Xu et al. 1999; 5, Mo et al. 1997; 6, ten Berge et al. 1998; 7, Juriloff et al. 1992; 8, McLeod et al. 1980; 9, Qui et al. 1997; 10, Schorle et al. 1996; 11, Zhang et al. 1996; 12, Lyon et al. 1996; 13, Curry 1959; 14, Arikawa-Hirasawa et al. 1999; 15, Satokata and Maas 1994; 16, Acampora et al. 1999b; 17, Depew et al. 1999; 18, Chisaka et al. 1992; 19, Gavalas et al. 1998; 20, Lufkin et al. 1991; 21, Mark et al. 1993; 22, Rossel and Cappechi 1999; 23, Gendron-Maguire et al. 1993; 24, Mallo 1997; 25, Mallo and Gridley 1996; 26, Rijli et al. 1993; 27, Hardisty et al., in preparation; 28, Kingsley et al. 1992; 29, J. Cable and Steel, in preparation; 30, Honarpour et al. 2000; 31, Naora et al. 1994; 32, Gavalas et al. 1998; 33, Goddard et al. 1996; 34, Rossel and Cappechi 1999; 35, Studer et al. 1996; 36, Barrow and Cappechi 1996; 37, Altschuler et al. 1991; 38, Bohne and Harding 1997; 39, Bonadio et al. 1990; 40, Dupé et al. 1999; 41, Ghyselinck et al. 1997; 42, Lohnes et al. 1994; 43, Peters et al. 1998; 44, Rivera-Pérez et al. 1995; 45, Yamada et al. 1995; 46, Zhu et al. 1997; 47, Kurihara et al. 1994; 48, Winograd et al. 1997; 49, Watanabe and Yamada 1999; 50, Acampora et al. 1999a; 51, Matsuo et al. 1995; 52, Morsli et al. 1999; 53, Minowa et al. 1999; 54, Phippard et al. 1999; 55 Phippard et al. 2000; 56, Theiler and Sweet 1986; 57, Clouthier et al. 1998; 58, Yanagisawa et al. 1998; 59, Martin et al. 1995; 60, Yang et al. 2000.

the gene, and a radiation-induced deletion of a similar upstream region has been found in the sex-linked fidget mouse mutant (de Kok et al. 1995; Phippard et al. 2000). Finally, the *p73* knockout recently reported is the first to show a single gene mutation conferring a predisposition to middle ear inflammation, and this is mediated by the gene’s involvement in the immune system (Yang et al. 2000). Since otitis media in children can be a serious clinical problem, the finding of a single gene predisposing to middle ear

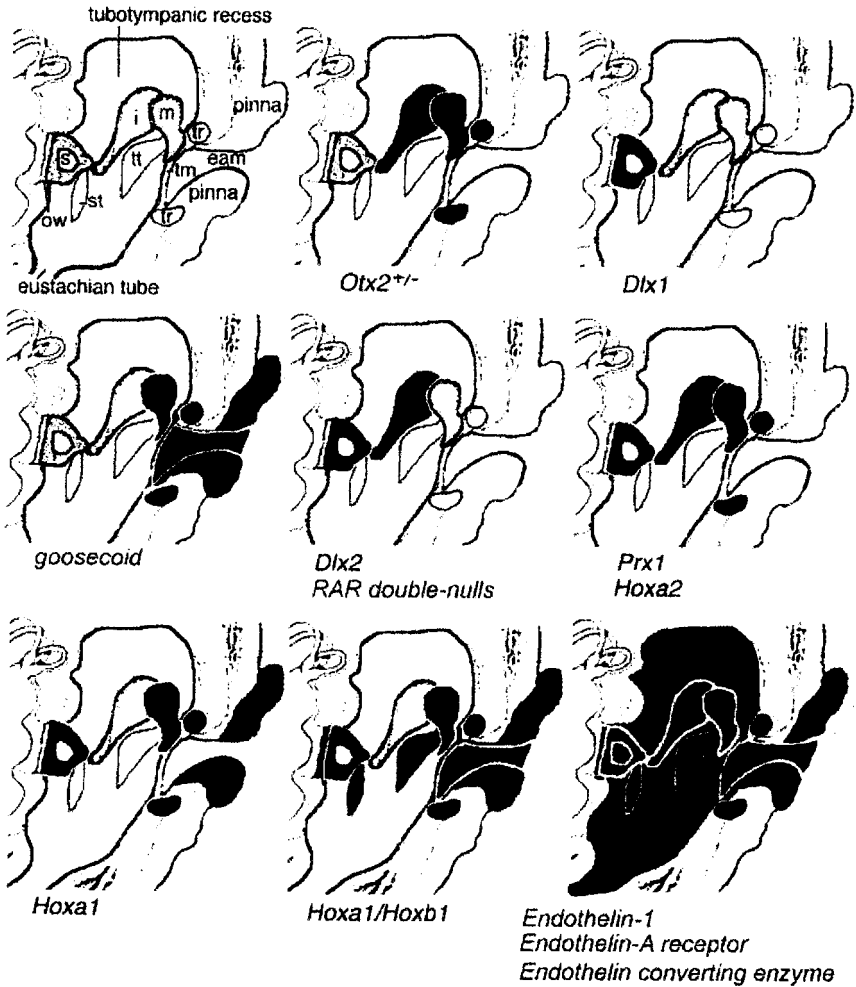


FIGURE 8.1. A schematic representation of the middle and outer ear showing the structures affected by mutations in various genes. The structures altered by the mutation are indicated in black. These alterations include duplications, hypomorphisms, or missing elements. The reported genes represent homozygous null mutants unless otherwise stated. eam = external auditory meatus, i = incus, m = malleus, ow = oval window, s = stapes, st = stapedius muscle, tm = tympanic membrane, tr = tympanic ring, tt = tensor tympani muscle. [Reprinted from Trends in Neuroscience Vol. 22, Fekete, DM, Development of the vertebrate ear: insights from knockouts and mutants, pp. 263–269, Copyright 1999, with permission from Elsevier Science.]

inflammation suggests a candidate gene that could be investigated for changes in children most severely affected.

2.2 Inner Ear Malformations

Gross malformations (morphogenetic defects) of the inner ear occur in many different mutants, as listed in Table 8.2. Many of these show semicircular canal defects, such as truncations of one or more of the canals (Fig. 8.2), and the preponderance of these may reflect ascertainment bias because they are relatively easy to detect. However, defects can range from a tendency for a single canal to be thin or truncated (Fig. 8.2B), to an extreme failure of the otic vesicle to develop into anything more than an elongated

TABLE 8.2. Mouse mutants with inner ear malformations

Mutant name	Symbol	Gene	Chromosome	Inheritance	Origin	Key references
Wocko	<i>Wo</i>	NK	1	D	I	1
Eyes absent 1 homologue KO	<i>Eya1</i>	<i>Eya1</i>	1 (10.4)	SD	T	2–4
Spotch	<i>Pax3</i>	<i>Pax3</i>	1 (44.0)	SD	S, R	5–9
	<i>Sp</i>					
Dreher	<i>dr, sst</i>	<i>Lmx1a</i>	1 (88.2)	R	S	10, 11
	<i>Lmx1a</i>					
Looptail	<i>Lp</i>	NK	1 (93.7)	SD	S	5, 12, 13
Prx2 KO	<i>Prx2, S8</i>	<i>Prx2</i>	2 (19)	R, E	T	14
Fidget	<i>fi</i>	NK	2 (34.0)	R	S	15
Distal-less	<i>Dlx2</i>	<i>Dlx2</i>	2 (44)	R, E	T	16
homeobox 2 KO						
Kreisler	<i>kr Krlm</i>	<i>Krml</i>	2 (91.0)	R	R, C	17–23
	<i>MafB</i>					
Wheels	<i>Whl</i>	NK	4	D	C	26, 27
Maloney sarcoma oncogene KO	<i>Mos</i>	<i>Mos</i>	4 (0)	SD	T	28, 29
Sightless	<i>Sig</i>	NK	6 (1)	SD	R	30–34
<i>Dlx5</i> KO	<i>Dlx5</i>	<i>Dlx5</i>	6 (2)	R	T	35, 36
Homeobox A1 KO	<i>Hoxa1</i>	<i>Hoxa1</i>	6 (26.3)	R, E	T	37–41
Homeobox A2 KO	<i>Hoxa2</i>	<i>Hoxa2</i>	6 (26.3)	R	T	42–45
Nijmegen waltzer	<i>nv</i>	NK	7 (4.2)	R	S	46, 47
Fibroblast growth factor receptor 2, isoform IIIb, KO	<i>Fgfr2</i>	<i>Fgfr2</i>	7 (63)	R	T	48
<i>Hmx3</i> KO	<i>Hmx3</i>	<i>Hmx3</i>	7 (65)	R, E	T	49, 50
	<i>Nkx5.1</i>					
<i>Hmx2</i> KO	<i>Hmx2</i>	<i>Hmx2</i>	7 (65)	R, E	T	51
	<i>Nkx5.2</i>					
Head bobber	<i>hb</i>	NK	7 (65)	R	I	52
Fibroblast growth factor 3 KO	<i>Fgf3, int-2</i>	<i>Fgf3</i>	7 (72.4)	R	T	53

TABLE 8.2. *Continued*

Mutant name	Symbol	Gene	Chromosome	Inheritance	Origin	Key references
Apoptotic protease activating factor 1 KO	<i>Apaf1</i>	<i>Apaf1</i>	10	R	T	54
Histidinaemia	<i>his</i> <i>hal</i>	<i>his</i>	10 (51)	R, M	S	55, 56
Orthodenticle homologue 1 KO	<i>Otx1</i>	<i>Otx1</i>	11 (12)	R, E	T	57–60
Neurofibromatosis 1 KO	<i>Nf1</i>	<i>Nf1</i>	11 (46)	R, E	T	61
<i>Cola1</i> KO	<i>Cola1</i> , <i>Mov-13</i>	<i>Cola1</i>	11 (56)	NK	T	27, 62, 63
Homeobox B1 KO	<i>Hoxb1</i>	<i>Hoxb1</i>	11 (56)	R, E	T	64–67
Retinoic acid receptor α KO	<i>Rara</i>	<i>Rara</i>	11 (57.8)	R, E	T	68–70
Extra toes	<i>Xt</i> <i>Gli3</i>	<i>Gli3</i>	13 (8)	SD	S	71, 72
RAS p21 protein activator KO	<i>Rasa</i> , <i>Gap</i>	<i>Rasa</i>	13 (47)	R, E	T	61
Bone morphogenetic protein 4 KO	<i>Bmp4</i>	<i>Bmp4</i>	14	SD	T	73
Retinoic acid receptor β KO	<i>Rarb</i>	<i>Rarb</i>	14 (1.5)	R, E	T	68–70
Orthodenticle homologue-2 KO	<i>Otx2</i>	<i>Otx2</i>	14 (19)	SD, E	T	59, 60, 74
Disproportionate micromelia	<i>Dmm</i> <i>Col2a1</i>	<i>Col2a1</i>	15 (54.5)	SD	R	75–77
Retinoic acid receptor γ	<i>Rarg</i>	<i>Rarg</i>	15 (57.4)	R, E	T	70
Punk rocker, <i>Isk</i> KO	<i>Isk</i> , <i>Kcne1</i> <i>pkc</i>	<i>Kcne1</i>	16 (64.4)	R	T	78, 79
Fused	<i>Fu</i> , <i>Ki</i> , <i>Kb</i> <i>Axin</i>	<i>Axin</i>	17 (11.8)	SD, M	S, C, I	80, 81
Twirler	<i>Tw</i>	NK	18 (3)	SD	S, I?	82, 83
Shaker-with-syndactylism	<i>sy</i> , <i>Nkcc1</i> , <i>Slc12a2</i> , <i>mBSC2</i>	<i>Slc12a2</i>	18 (27)	R	R, S, C T	84–89
Dancer	<i>Dc</i>	NK	19 (6)	SD	S	90–92
Kidney and retinal defects; <i>Pax2</i> KO	<i>krd</i> <i>Pax2</i>	<i>Pax2</i>	19 (43)	SD	T, S, I	93–95
Sex-linked fidget <i>Pou3f4</i> KO	<i>slf</i> , <i>Brn4</i> <i>Pou3f4</i>	<i>Pou3f4</i>	X (48.4)	SD	T, R	96–98
Jackson waltzer	<i>jv</i>	NK	NK	R	S	34
Porcine tail	<i>pr</i>	NK	NK	R	S	99
Pivoter	<i>Pv</i>	NK	NK	SD	S	34
Rotating	<i>rg</i>	NK	NK	R	S	100
Zigzag		NK	NK	E	S	101, 102
Shaker-short	<i>st</i>	NK	NK	R	S	103
Waltzer-type	<i>Wt</i>	NK	NK	SD	S	104, 105
Netrin 1 KO	<i>Netrin1</i> <i>Ntn1</i>	<i>Ntn1</i>	NK	R	T	106

TABLE 8.2. *Continued*

Mutant name	Symbol	Gene	Chromosome	Inheritance	Origin	Key references
Retinaldehyde dehydrogenase2 KO	<i>Raldh2</i>	<i>Raldh2</i>	NK	R	T	107
Retinoic acid receptor χ KO	<i>Rarc</i>	<i>Rarc</i>	NK	R, E	T	70
<i>Prx1</i> KO	<i>Prx1</i>	<i>Prx1</i>	NK	R, E	T	14, 108
	<i>Mhox</i>					
Forkhead homologue 10 KO	<i>Fkh10</i>	<i>Fkh10</i>	NK	R	T	109

The table includes the major genes or loci known to be involved in deafness and/or balance defects. Only the key references describing the ear phenotype and the initial identification of the gene are included, so the list is not intended to be comprehensive. The chromosomal localization is given where this is known, with the distance in cM from the centromere given in parentheses. KO, Knock-out. NK, Not known. Under Inheritance column: R, Recessive; D, Dominant; SD, Semidominant; E, Epistatic; M, Maternal effect. Under Origin column: R, Radiation-induced; S, Spontaneous; T, Transgenics and knock-outs; C, Chemical mutagenesis; I, Transgenic Insertional mutations.

References: 1, Crenshaw et al. 1991; 2, Abdelhak et al. 1997; 3, Johnson et al. 1999; 4, Xu et al. 1999; 5, Deol 1966a; 6, Epstein et al. 1991; 7, Fleming et al. 1996; 8, Goulding et al. 1993; 9, Steel and Smith 1992; 10, Deol 1964a; 11, Millonig et al. 2000; 12, Wilson 1985; 13, Wilson and Wyatt 1995; 14, ten Berge et al. 1998; 15, Truslove 1956; 16, Qiu et al. 1997; 17, Cordes and Barsh 1994; 18, Deol 1964b; 19, Eichman et al. 1997; 20, Frohman et al. 1993; 21, McKay et al. 1994; 22, McKay et al. 1996; 23, Ruben 1973; 24, Schorle et al. 1996; 25, Zhang et al. 1996; 26, Nolan et al. 1995; 27, Bohne and Harding 1997; 28, Propst et al. 1990; 29, Rauch 1992; 30, Deol 1976; 31, Deol 1980; 32, Deol 1983; 33, Khaze'i 1974; 34, Lyon et al. 1996; 35, Acampora et al. 1999b; 36, Depew et al. 1999; 37, Chisaka et al. 1992; 38, Gavalas et al. 1998; 39, Lufkin et al. 1991; 40, Mark et al. 1993; 41, Rossel and Cappechi 1999; 42, Gendron-Maguire et al. 1993; 43, Mallo 1997; 44, Mallo and Gridley 1996; 45, Rijli et al. 1993; 46, Deol 1974; 47, Van Abeelen and Van Der Kroon 1967; 48, De Moerloose et al. 2000; 49, Hadrys et al. 1998; 50, Wang et al. 1998; 51, Chan et al. 2000; 52, Hardisty et al., in preparation; 53, Mansour et al. 1993; 54, Honarpour et al. 2000; 55, Kacer et al. 1979; 56, Taylor et al. 1993; 57, Acampora et al. 1996; 58, Acampora et al. 1998; 59, Acampora et al. 1999a; 60, Morsli et al. 1999; 61, Henkemeyer et al. 1995; 62, Altschuler et al. 1991; 63, Bonadio et al. 1990; 64, Gavalas et al. 1998; 65, Goddard et al. 1996; 66, Rossel and Cappechi 1999; 67, Studer et al. 1996; 68, Dupé et al. 1999; 69, Ghyselinck et al. 1997; 70, Lohnes et al. 1994; 71, Hui and Joiner 1993; 72, Johnson 1967; 73, Teng et al. 2000; 74, Matsuo et al. 1995; 75, Berggren et al. 1997; 76, Pace et al. 1997; 77, Van De Water and Galinovic-Schwartz 1987; 78, Vetter et al. 1996; 79, Letts et al. 2000; 80, Deol 1966b; 81, Zeng et al. 1997; 82, Lyon 1958; 83, Ting et al. 1994; 84, Delpire et al. 1999; 85, Deol 1963; 86, Deol 1968; 87, Dixon et al. 1999; 88, Flagella et al. 1999; 89, Johnson et al. 1998; 90, Deol and Lane 1966; 91, Wenngren and Anniko 1988; 92, Wenngren and Anniko 1990; 93, Favor et al. 1996; 94, Keller et al. 1994; 95, Torres et al. 1996; 96, Minowa et al. 1999; 97, Phippard et al. 1999; 98, Phippard et al. 2000; 99, McNutt 1968; 100, Deol and Dickie 1967; 101, Lyon 1960; 102, Deol 1980; 103, Bonnevie 1936; 104, Deol 1976; 105, Stein and Huber 1960; 106, Salminen et al. 2000; 107, Niederreither et al. 2000; 108, Martin et al. 1995; 109, Hulander et al. 1998.

cyst (Fig. 8.2D). Auditory function may be undetectable in some of these mutants, or may be normal in mutants with only minor vestibular malformations, but in many cases the only test of hearing has been the Preyer reflex, which cannot be taken to indicate normal thresholds for cochlear

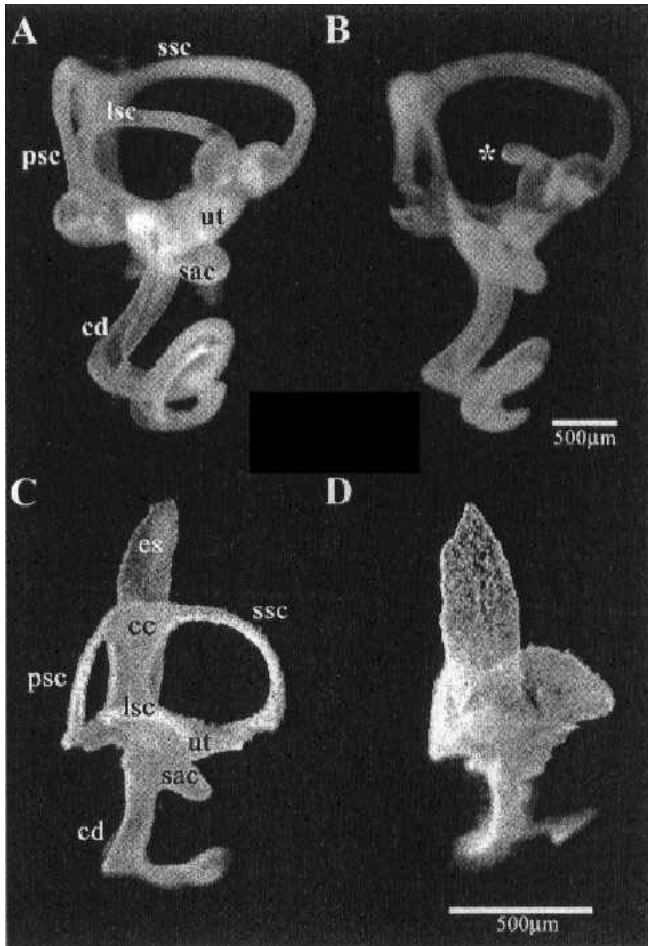


FIGURE 8.2. Paint-filled inner ears showing two types of malformation. (A) and (C) are controls at the same age as the mutants (B) and (D), respectively. (B) This mutant, one day after birth, shows a truncation of the lateral canal, shown by the asterisk. (D) The mutant represented, embryonic day 14.5, shows complete lack of development of the semicircular canals. The vestibular part of the inner ear remains as a cyst-like structure, and the endolymphatic sac (es) is enlarged. ssc, superior semicircular canal; psc, posterior semicircular canal; lsc, lateral semicircular canal; ut, utricle; sac, sacculle; cd, cochlear duct; es, endolymphatic sac; cc, crus commune.

activity (Kiernan et al. 1999). Furthermore, as for outer and middle ear defects, many of the knock-out mutants show early death, precluding assessment of cochlear function by most standard physiological approaches; cochlear function can be first detected at around twelve days after birth in normal mice.

The inner ear develops from a thickening of the surface ectoderm at the side of the developing head, called the otic placode. This placode invaginates into a pit that closes off from the surface to form the otic vesicle or otocyst, lying just lateral to the neural tube. At first an ovoid shape, this otocyst begins to develop into the characteristic shape of the mature inner ear (Fig. 8.2). The endolymphatic duct is the first structure to protrude, from the dorsomedial region of the otocyst. Several mutants, such as *kreisler* and the *Fgf3* and *Hoxa1* knockout mutants, fail to form a normal endolymphatic duct, and subsequent development of the inner ear is severely abnormal. Much of the dorsal region of the otic vesicle forms the vestibular part of the ear, while the ventral tip extends and coils round to form the cochlea. Semicircular canals form from flattened semicircular pouches extending from the dorsal region of the otic vesicle. The central regions of these pouches meet in the middle and the cells there move away to leave an open rim around the edge, the semicircular canal (Martin and Swanson 1993). The lateral canal is the last to form, which might explain why it is the only canal to be affected, or is the most commonly affected canal, in some mouse mutants as well as in humans. The perilymphatic compartments form around the shape of the otic vesicle, by the resorption of mesenchymal cells to leave fluid-filled channels, and the bony labyrinth forms around this template by condensation of mesenchyme to form cartilage, followed by bone deposition.

What is it that controls the development of the complex form of the labyrinth? It has long been thought that surrounding tissues, particularly the neural tube, have a role in patterning the otic vesicle, helping to determine the fate of each part (Deol 1966a; Torres and Giraldez 1998). Recent observations that mutation of some genes that are not normally expressed in the otic vesicle can still lead to severe inner ear malformations provides striking evidence that this is indeed the case. An example of this is the *Hoxa1* gene, which is expressed in the adjacent neural tube, but not in the otic vesicle: knockout of this gene leads to severe inner ear malformation, presumably by a failure of the neural tube in the mutant to provide the correct inductive signals to pattern the otic vesicle (Chisaka et al. 1992; Lufkin et al. 1991). Fekete (1996) has proposed a model to explain how the surrounding tissues might influence the creation of compartments within the otocyst. These compartments are marked by expression of various genes in restricted regions of the otic epithelium. Of course, mutations in the genes that are expressed in the otocyst can also lead to abnormal inner ear morphogenesis. Several genes that are implicated in morphogenetic defects of the inner ear are expressed in the otic epithelium in positions that correlate reasonably well with the defect seen. For example, *Pax2* is expressed in the ventral otic vesicle, and mutation of the gene leads to absence of the cochlea (Favor et al. 1996; Torres et al. 1996), while *Hmx3* is expressed in the dorsal otic vesicle, and knockout of this gene leads to gross malformation of the vestibule (Hadrys et al. 1998; Wang et al. 1998).

A glance at Table 8.2 shows that many of the known genes underlying inner ear malformations are transcription factors, which is not surprising given that these defects must arise very early in development. However, one surprising recent finding has been that genes involved in ionic homeostasis of the ear can be involved in the pathology. For example, the shaker-with-syndactylylism mutant was originally reported as showing thin semicircular canals (Deol 1963), but this defect is now known to result from mutation of the *Slc12a2* gene, encoding a cotransporter implicated in ion transport in marginal cells of the stria vascularis (Dixon et al. 1999). All endolymphatic compartments collapse to some extent around the time of birth in this mutant, including the lumen of each semicircular canal, suggesting failure of normal endolymph production (Deol 1963). It seems likely that the narrower template of a collapsed lumen leads to the formation of a thinner canal as the cartilage is laid down around it (Dixon et al. 1999).

Several of the mutants in this group have human homologues. Human genes are symbolized in capitals, and mouse genes are in lower-case letters, but otherwise they generally have the same symbol. The *Eya1* and *Pou3f4* mouse mutants show broadly similar malformations, as seen in the human syndromes branchio-oto-renal syndrome (with mutations in the human *EYAI* gene) and X-linked mixed deafness with gusher (mutations in the human *POU3F4* gene), respectively (Griffith and Friedman, Chapter 6). In particular, the wide internal auditory meatus observed in X-linked mixed deafness with gusher, which is believed to allow direct communication of perilymph with cerebrospinal fluid, is also seen in the mouse mutant. The third example is the histidinaemia mouse mutant, in which a mutation in the histidase gene leads to an excess of histidine in the mother, which in turn seems to have a teratogenic effect on the developing fetus, causing mild inner ear malformations (Kacser et al. 1979). Similar malformations have not been reported in humans suffering from maternal histidinaemia, although speech defects have been described. *Pax3* mutations in splotch mutant mice lead to malformations of the inner ear in homozygotes (Deol 1966a), but these mice die before birth with gross neural tube defects. A single human homozygous for a *PAX3* mutation survived and has been described as deaf with white skin and hair, but it is not known whether the child had malformed inner ears (Zlotogora et al. 1995). Mutations in several other genes in this group in humans, including *NFI*, *COLAI*, and *ISK*, can be associated with hearing impairment, but it is not known whether any inner ear malformation is present in affected people.

2.3 Neuroepithelial Defects

Neuroepithelial defects involve a primary abnormality in the neuroepithelia of the inner ear, such as the organ of Corti in the cochlea, the maculae of the saccule and utricle, and the cristae of the ampullae at the end of each

semicircular canal. These defects can affect the determination of cell fate in the neuroepithelium, the differentiation of cell types, the sculpting of the tissues as they develop, or the function of the various cell types, including hair cells. Table 8.3 lists the mouse mutants with this type of abnormality.

TABLE 8.3. Mouse mutants with neuroepithelial defects

Mutant name	Symbol	Gene	Chromosome	Inheritance	Origin	Key references
Integrin $\alpha 8$ KO	<i>Itga8</i>	<i>Itga8</i>	2	R	T	1
Activating transcription factor 2 KO	<i>Atf2</i> , <i>Creb2</i>	<i>Atf2</i>	2	R	T	2
Modifier of tubby hearing 1	<i>Moth1</i>	NK	2 (69)	R	S	3
Tailchaser	<i>Tlc</i>	NK	2 (77)	SD	C	4
Varitint-waddler	<i>Va</i>	NK	3 (74.8)	SD	S	5–7
Maloney sarcoma oncogene KO	<i>Mos</i>	<i>Mos</i>	4 (0)	SD	T	8, 9
Whirler	<i>wi</i>	NK	4 (31.4)	R	S	10
Jerker	<i>je</i>	NK	4 (80.1)	R	S	11–13
Fibroblast growth factor receptor 3 KO	<i>Fgfr3</i>	<i>Fgfr3</i>	5 (20)	R	T	14
Pirouette	<i>pi</i>	NK	5 (40)	R	S	15, 16
Nicotinic acetylcholine receptor $\alpha 9$ KO	<i>Acra9</i>	<i>Acra9</i>	5 (41)	R	T	17
Bronx waltzer	<i>bv</i>	NK	5 (63)	R	S	18–29
Atonal homolog 1 KO	<i>Atoh1</i> , <i>Math1</i>	<i>Atoh1</i>	6 (30)	R	T	30
Deafwaddler, Wriggle mouse Sagami, <i>Pmca2</i> KO	<i>dfw</i> , <i>wri</i> , <i>Pmca2</i> , <i>Atp2b2</i>	<i>Atp2b2</i>	6 (49.5)	R, SD	S	31–33
Cyclin dependent kinase inhibitor 1B KO	<i>Cdkn1b</i> <i>p27^{Kip1}</i>	<i>Cdkn1b</i>	6 (62)	R	T	34, 35
Cartilage matrix deficiency	<i>cmd</i> , <i>Agc</i>	<i>Agc</i>	7 (39)	R		36, 37
Shaker1	<i>Myo7a</i> , <i>sh1</i>	<i>Myo7a</i>	7 (48.1)	R	S, C	38–47
Tubby	<i>tub</i> , <i>CBT9</i>	<i>tub</i>	7 (51.45)	R	S	48–51
Snell's waltzer	<i>sv</i> , <i>Myo6</i>	<i>Myo6</i>	9 (44)	R	S, R	52–54
Spinner	<i>sr</i>	NK	9 (64)	R	S	55
Waltzer, Bustling	<i>v</i> , <i>bus</i>	NK	10 (30.3)	R	S, C	56–61
Modifier of deafwaddler	<i>mdfw</i>	NK	10 (30.3)	R, E	S	62
Age-related hearing Loss	<i>Ahl</i>	NK	10 (33)	R	S	63–66
Ames waltzer	<i>av</i>	NK	10 (40.2)	R	S	67, 68
Mocha	<i>mh</i> , <i>Ap3d</i>	<i>Ap3d</i>	10 (43)	R, M	S	69–71
Shaker2	<i>sh2</i> , <i>Myo15</i>	<i>Myo15</i>	11 (33.9)	R	R, S	72–74
Jackson shaker	<i>js</i>	NK	11 (77)	R	S	75–78

TABLE 8.3. *Continued*

Mutant name	Symbol	Gene	Chromosome	Inheritance	Origin	Key references
Hypothyroidism	<i>hyt, pet, Tshr</i>	<i>Tshr</i>	12 (37)	R	S	79–81
Jagged2 KO	<i>Jag2, sm, Serh</i>	<i>Jag2</i>	12 (57.9)	R	T	82
Homeobox, msh-like 2	<i>Msx2, Hox8</i>	<i>Msx2</i>	13 (32)	D	T	83
Neurogenin1 KO	<i>Ngn1, Neurod3</i>	<i>Neurod3</i>	13 (35)	R	T	84, 85
Dreidel, <i>Pou4f3</i> KO	<i>Pou4f3, ddl, Brn3.1, Brn3c</i>	<i>Pou4f3</i>	18 (24)	R	S, T	86–89
Deafness	<i>dn</i>	NK	19 (15)	R	S	90–96
Dystrophin	<i>Dmd, mdx</i>	<i>Dmd</i>	X (32)	R	S, C	97
Gyro, Hypophosphataemia	<i>Gy, Hyp, Phex, Pex</i>	<i>Phex</i>	X (65.4)	SD	R, S	98–103
Thyroid hormone receptor α	<i>Thra</i>	<i>Thra</i>		E	T	104
Thyroid hormone receptor β	<i>Thrb</i>	<i>Thrb</i>		R, E	T	104–106
<i>erbB4</i> receptor tg	<i>erbB4</i>	<i>erbB4</i>		D	T	107

The table includes the major genes or loci known to be involved in deafness and/or balance defects. Only the key references describing the ear phenotype and the initial identification of the gene are included, so the list is not intended to be comprehensive. The chromosomal localization is given where this is known, with the distance in cM from the centromere given in parentheses. KO, Knock-out. NK, Not known. Under Inheritance column: R, Recessive; D, Dominant; SD, Semidominant; E, Epistatic; M, Maternal effect. Under Origin column: R, Radiation-induced; S, Spontaneous; T, Transgenics and knock-outs; C, Chemical mutagenesis; I, Transgenic Insertional mutations.

References: 1, Littlewood Evans and Müller 2000; 2, Reimold et al. 1996; 3, Ikeda et al. 1999; 4, Kiernan et al. 1999; 5, Cable and Steel 1998; 6, Deol 1954; 7, Mikaelian et al. 1965; 8, Propst et al. 1990; 9, Rauch 1992; 10, Lane 1963; 11, Deol 1954; 12, Sjöström and Anniko 1992; 13, Steel and Bock 1983; 14, Colvin et al. 1996; 15, Deol 1956a; 16, Sidman et al. 1966; 17, Vetter et al. 1999; 18, Bock et al. 1982; 19, Demêmes and Sans 1985; 20, Deol 1981; 21, Deol and Gluecksohn-Waelsch 1979; 22, Horner et al. 1985; 23, Keithley and Feldman 1983; 24, Lenoir and Pujol 1984; 25, Schrott et al. 1989; 26, Sobkowicz et al. 1999; 27, Tucker et al. 1999; 28, Whitlon and Sobkowicz 1991; 29, Whitlon et al. 1996; 30, Bermingham et al. 1999; 31, Kosel et al. 1998; 32, Street et al. 1998; 33, Takahashi and Kitamura 1999; 34, Chen and Segil 1999; 35, Löwenheim et al. 1999; 36, Watanabe et al. 1994; 37, Yoo et al. 1991; 38, Deol 1956a; 39, Gibson et al. 1995; 40, Hasson et al. 1997; 41, Kikuchi and Hilding 1965; 42, Mburu et al. 1997; 43, Mikaelian and Ruben 1964; 44, Richardson et al. 1997; 45, Self et al. 1998; 46, Shnerson et al. 1983; 47, Steel and Harvey 1992; 48, Kleyn et al. 1996; 49, Noben-Trauth et al. 1996; 50, Ohlemiller et al. 1995; 51, Ohlemiller et al. 1997; 52, Avraham et al. 1995; 53, Deol and Green 1966; 54, Self et al. 1999; 55, Deol and Robins 1962; 56, Deol 1956a; 57, Deol 1956b; 58, Deol 1974; 59, Mikaelian et al. 1965; 60, Otani et al. 1995; 61, Yonezawa et al. 1996; 62, Noben-Trauth et al. 1997; 63, Erway et al. 1996; 64, Johnson et al. 1997; 65, Willott and Erway 1998; 66, Bohne and Harding 1997; 67, Alagrammam et al. 1999; 68, Osako and Hilding 1971; 69, Kantheti et al. 1998; 70, Lane and Deol 1974; 71, Rolfson and Erway 1984; 72, Deol 1954; 73, Probst et al. 1998; 74, Sobin et al. 1982; 75–77, Kitamura et al. 1991a, b, c; 78, Kitamura et al. 1992; 79, Bertoni et al. 1993; 80, O'Malley et al. 1995; 81, Stein et al. 1994; 82, Lanford et al. 1999; 83, Hoffman et al. 1995; 84, Ma et al. 1998; 85, Fritzsche et al. 1999a; 86, Erkman et al. 1996; 87, Xiang et al. 1997; 88, Xiang et al. 1998; 89, W. Frankel pers. comm. 2000; 90, Bock and Steel 1983; 91, Deol and Kocher 1958; 92, Durham et al. 1989; 93, Horner et al. 1985; 94, Pujol et al. 1983; 95, Steel and Bock 1980; 96, Steel and Bock 1984; 97, Raynor and Mulroy 1997; 98, Eicher et al. 1976; 99, Lyon et al. 1986; 100, Meyer et al. 1998; 101, Popelka et al. 1986; 102, Steel et al. 1989; 103, Strom et al. 1997; 104, Rüschi et al. 1998; 105, Campos Barros et al. 1998; 106, Forrest et al. 1996; 107, Rio et al. 1999.

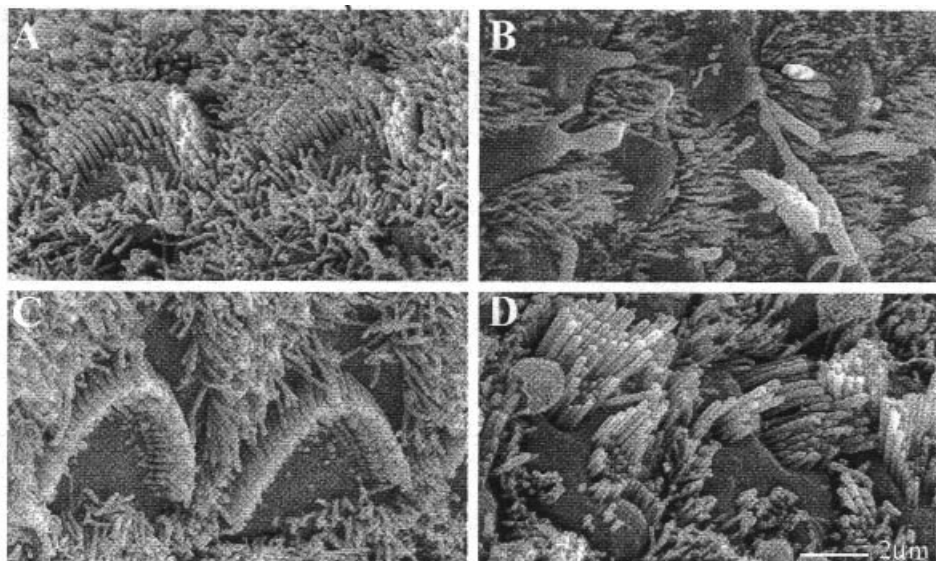


FIGURE 8.3. Scanning electron micrographs showing two types of hair cell abnormality. (B) Outer hair cells of Snell's waltzer (*Myo6^{sv}/Myo6^{sv}*) mutants at 7 days after birth, in which the stereocilia are disorganized and fused together. (D) Outer hair cells of the shaker1 (*Myo7a^{816SB}/Myo7a^{816SB}*) mutant at 3 days after birth. The stereocilia are not arranged in the characteristic W-shape seen in controls, but instead clump together in clusters on the hair cell surface. (A) and (C) show littermate controls.

Several of the mutants show abnormal differentiation of sensory hair cells. The shaker1, tailchaser, and waltzer mutants all have disorganized hair bundles around the time of birth (Fig. 8.3) (Self et al. 1998; Kiernan et al. 1999; Yonezawa et al. 1996; R. Holme, personal communication 2000). In these mutants, hair cells are present in their normal arrangement in the organ of Corti, and they extend many microvilli from their upper surface, which develop into stereocilia. However, the normal V-shaped arrangement of stereocilia within each bundle either does not form correctly, or becomes progressively disorganized. Hair cells eventually degenerate, as is generally the case in hearing-impaired mutants, but it is clear from the timing that this must be secondary to the early abnormalities in hair cell development. This emphasizes the importance of studying development in a model species to determine the primary defect.

A different hair bundle defect is observed in Snell's waltzer mutants. In these hair cells, adjacent stereocilia fuse together, starting from the base near the insertion into the cuticular plate of the hair cell at around the time of birth, and forming giant protrusions from the top of each cell within a few days (Self et al. 1999). A third type of stereocilia defect is seen in the

shaker2 mutant. In these mice, the stereocilia are all shorter than normal, although they are arranged in the normal V-shape on top of each hair cell (Probst et al. 1998). Again, hair cells ultimately die in both Snell's waltzer and shaker2 mutants.

The shaker1, Snell's waltzer and shaker2 genes have all been identified by positional cloning, and interestingly they all involve unconventional myosin genes, *Myo7a*, *Myo6*, and *Myo15* respectively. These three myosins obviously have important, but distinctive, roles in the development and maintenance of stereocilia bundles on hair cells. On the basis of its distribution along the cell membrane covering each stereocilium, it has been suggested that myosin VIIa may play a role in cross-linking stereocilia, possibly by transporting or anchoring the extracellular link material (Hasson et al. 1997). Myosin VI, on the other hand, might be important in anchoring the cell membrane between adjacent stereocilia to the actin-rich cuticular plate just below the apical surface of the hair cell; this could prevent the natural tendency of lipid membranes in water to adopt the lowest possible surface area, which would lead to the observed "zipping up" of these membranes in the mutants (Self et al. 1999).

Two mutants demonstrate little or no development of sensory hair cells. The *Math1* knockout mutant shows no sign of development of hair cells within the organ of Corti (Bermingham et al. 1999), suggesting that the gene is essential for differentiation of these cells. It is the earliest gene known to be involved in the cascade of gene activity that results in a differentiated hair cell, and is thus of much interest to those working on hair cell regeneration. The *Pou4f3* knockout mutant also shows very little differentiation of hair cells (Erkman et al. 1996; Xiang et al. 1997), but expression studies show that early hair cell markers like *Myo7a* are expressed in cells in the position of putative hair cells, indicating that differentiation of the hair cell phenotype does begin in these mutants (Xiang et al. 1998). *Pou4f3* must therefore act later in hair cell differentiation than *Math1*.

Two genes have been shown to have a role in cell fate determination or proliferation in the organ of Corti. *Jagged2* knockout mutants have an increased number of hair cells (Lanford et al. 1999). This gene is known to be involved in Notch/Delta signalling, so this observation supports the proposal that lateral inhibition mediated by Notch/Delta homologues plays a role in determining whether a cell becomes a hair cell or a supporting cell. Because supporting cells did develop in the *Jagged2* mutants, this suggests that *Jagged2* is not the only Notch ligand involved in the process of deciding cell fate, but that other factors must also be involved. The second gene in this category is *p27^{Kip1}* (Chen and Segil 1999; Löwenheim et al. 1999). In mutants with this gene inactivated, cell proliferation continues beyond the normal time of cell division in the organ of Corti, leading to additional cells being formed.

The bronx waltzer mutant has a unique cochlear pathology. Most inner hair cells and vestibular hair cells die from around 17.5 days of gestation,

while outer hair cells remain intact into adulthood (Deol and Gluecksohn-Waelsch 1979; Whitlon et al. 1996). This gene has not yet been identified, although positional cloning is underway (Bussoli et al. 1997). It is the first example of a gene essential for the continued survival of one hair cell type, but not another; it is of particular interest because inner hair cells receive most of the afferent innervation of the cochlea.

Lastly, supporting cells can also be involved directly in the pathology of deafness. For example, the *Fgfr3* knockout mutant has a primary effect upon pillar cell development, which results in impaired hearing (Colvin et al. 1996). This mutant and others in which specialized cell types around the cochlear duct are affected emphasize the importance of the correct function of the whole system, and demonstrate that hair cells alone are not sufficient to ensure normal auditory function.

Several of the genes involved in mouse neuroepithelial defects are also involved in human deafness. Usher syndrome type 1B, atypical Usher syndrome, and two forms of recessive (DFNB2) and dominant (DFNA11) non-syndromic deafness are all due to mutations in the *MYO7A* gene (Weil et al. 1995, 1997; Liu et al. 1997a,b), and so are likely to show similar hair cell defects as in the shaker1 mutants. The limited observations of temporal bone pathology in Usher syndrome patients is consistent with a neuroepithelial defect, but no scanning electron microscopy of very early stages of hair bundle development has been carried out in people with *MYO7A* mutations, nor is it ever likely to be. Both mice and humans with *Myo7a/MYO7A* mutations show hearing impairment and also balance defects. However, humans with Usher syndrome have the additional feature of retinitis pigmentosa, but so far only minor differences in retinal function have been demonstrated in shaker1 mice, with no overt retinal degeneration (Liu et al. 1998, 1999; Hasson et al. 1997). The reason for this difference is not clear, but may be related to the shorter lifespan of mice compared with humans, or may be due to differences in the genetic background, since some humans with *MYO7A* mutations also escape retinal degeneration.

A form of recessive nonsyndromic deafness (DFNB3) found in an isolated population in Bali is associated with mutation of the *MYO15* gene (Wang et al. 1998), but there is no histopathology to assess whether the affected people have short stereocilia, like the shaker2 mouse mutants. There is an indication that they have a balance problem, similar to the mouse homologue.

The *POU4F3* gene underlies a dominant form of late-onset, progressive hearing loss in humans with DFNA15 (Vahava et al. 1998). In mice heterozygous for the knockout allele of *Pou4f3*, there is no obvious progressive hearing loss, but again absolute age or genetic background effects might explain this difference, or possibly the human mutation has a dominant negative effect. Mice that are homozygous for the knockout mutation show very little development of sensory hair cells, and the observation of late-

onset hearing loss in humans suggests that this transcription factor may also have a role in hair cell maintenance.

2.4 *Abnormal Endolymph Homeostasis*

A number of the genes involved in deafness in humans appear likely to have a role in maintaining the homeostasis of the endolymph of the cochlear duct (eg Steel 1999 Table 8.4), which emphasizes the importance of the properties of endolymph in allowing hair cells to function normally. Endolymph has an unusual ionic composition, with high potassium and low sodium levels, and is maintained at a high positive resting potential (endocochlear potential, EP) of around 100 mV in a mouse. The high positive potential provides a large potential difference across the top of the hair cell, from the positive endolymph to the negative interior of the hair cell, which presumably aids cation flow through the transduction channels. The predominance of potassium in the endolymph means that a large proportion of the transduction current that passes through the transduction channel is potassium rather than sodium, and a large influx of sodium would probably have a deleterious effect on the cell. The high-potassium, low-sodium levels are present at birth, but the EP develops postnatally, from around 10 to 20 mV at six days to adult levels by around two weeks of age (Steel and Barkway 1989). The stria vascularis on the lateral wall of the cochlear duct is primarily responsible for generating the EP and (presumably) the ionic composition of endolymph. The stria is believed to generate EP by electrogenic pumping of potassium into the endolymph.

The stria vascularis has an abundant supply of blood vessels, but the potassium pumped into the cochlear duct is not immediately derived from blood (Marcus 1986; Salt et al. 1987). There are several lines of circumstantial evidence that potassium may be recycled around the cochlear duct (Fig. 8.4). This was first proposed after careful ultrastructural study revealed an extensive network of gap junctions that link supporting cells of the organ of Corti together, and fibrocytes of the spiral ligament and spiral limbus to their neighbours (Kikuchi et al. 1995; Spicer and Schulte 1996). Connexin molecules form a core component of these gap junctions, and mutations in several gap-junction genes expressed in these tissues cause deafness in humans. The most common form of recessive human nonsyndromic deafness is due to mutations in the *GJB2* gene, encoding connexin 26 (Griffith and Friedman, Chapter 6), and this gene is widely expressed in gap junctions in the cochlea. Unfortunately, knockout of this gene causes very early embryonic lethality in mice because of an essential role in mouse placental development (Gabriel et al. 1998), unrelated to its function in the ear. No doubt conditional mutants will be constructed to allow investigation of its role in cochlear function.

The human and mouse mutations in genes expressed along the putative potassium recycling route allow piecing together of some of the steps

TABLE 8.4. Mouse mutants with defects in endolymph homeostasis

Mutant name	Symbol	Gene	Chromosome	Inheritance	Origin	Key references
Collagen 4A3 KO	<i>Col4a3</i>	<i>Col4a3</i>	1	R	T	1
Splotch	<i>Sp, Pax3</i>	<i>Pax3</i>	1 (44)	SD	S,R	2–6
Lethal spotting	<i>ls, Edn3</i>	<i>Edn3</i>	2 (104)	R	S, T	7–9
Endothelin-3 KO						
Varitint-waddler	<i>Va</i>	NK	3 (74.8)	SD	S	10–12
Light allele of brown	<i>B^{lt}, Tyrp1</i>	<i>Tyrp1</i>	4 (38)	SD	S, R	13,14
Mpv17	<i>Mpv17</i>	<i>Mpv17</i>	5	R	I	15–17
Dominant spotting	<i>Kit, W</i>	<i>Kit</i>	5 (42.0)	SD	S, R	18–33
Patch	<i>Ph, Pdgfra</i>	<i>Pdgfra?</i>	5 (42.0)	SD	S	22, 34
Homeobox A2 KO	<i>Hoxa2</i>	<i>Hoxa2</i>	6 (26.3)	R	T	35–38
Waved1	<i>wal, Tgfa</i>	<i>Tgfa</i>	6 (35.8)	R	S, T	39–41
Microphthalmia	<i>mi, Mitf, vit</i>	<i>Mitf</i>	6 (40)	R, SD	S, R, I	22, 24, 42–48
Steel	<i>Sl, Mgf</i>	<i>Mgf</i>	10 (57)	SD	S, R, I	22, 29, 31, 49–51
Piebald	<i>s, Ednrb</i>	<i>Ednrb</i>	14 (51)	R	S, R, T	24, 52–55
Punk rocker, <i>Isk</i> KO	<i>Isk, Kcne1</i> <i>pkr</i>	<i>Kcne1</i>	16 (64.4)	R	T	56, 57
Shaker-with-syndactylism	<i>sy, Slc12a2</i> <i>Nkcc1,</i> <i>mBSC2</i>	<i>Slc12a2</i>	18 (27)	R	R, S, C, T	53, 58–62
Lymphoproliferation, Fas antigen KO	<i>lpr, Fas</i>	<i>Fas</i>	19 (23)	R	S	63–66
<i>Pou3f4</i> KO, Sex-linked fidget	<i>Pou3f4,</i> <i>slf, Brn4</i>	<i>Pou3f4</i>	X (48.4)	SD	T, R	67–69
<i>Kcnq1</i> KO	<i>Kcnq1,</i> <i>Kv1qt1</i>	<i>Kcnq1</i>		R	T	70

The table includes the major genes or loci known to be involved in deafness and/or balance defects. Only the key references describing the ear phenotype and the initial identification of the gene are included, so the list is not intended to be comprehensive. The chromosomal localization is given where this is known, with the distance in cM from the centromere given in parentheses. KO, Knock-out. NK, Not known. Under Inheritance column: R, Recessive; D, Dominant; SD, Semidominant; E, Epistatic; M, Maternal effect. Under Origin column: R, Radiation-induced; S, Spontaneous; T, Transgenics and knock-outs; C, Chemical mutagenesis; I, Transgenic Insertional mutations.

References: 1, Cosgrove et al. 1998; 2, Deol 1966a; 3, Epstein et al. 1991; 4, Fleming et al. 1996; 5, Goulding et al. 1993; 6, Steel and Smith 1992; 7, Baynash et al. 1994; 8, Mayer and Maltby 1964; 9, Cable and Steel, unpublished observations; 10, Cable and Steel 1998; 11, Deol 1954; 12, Mikaelian et al. 1965; 13, Cable et al. 1993; 14, Johnson and Jackson 1992; 15, Meyer zum Gottesberge et al. 1996; 16, Müller et al. 1997; 17, Reuter et al. 1998; 18, Cable et al. 1992; 19, Cable et al. 1994; 20, Cable et al. 1995; 21, Chabot et al. 1988; 22, Deol 1970a; 23, Deol 1970b; 24, Deol 1971; 25, Fujimura et al. 1999; 26, Geissler et al. 1988; 27, Hibino et al. 1997; 28, Rask-Andersen 1987; 29, Schrott et al. 1990; 30, Schrott and Spoendlin 1987; 31, Schulte and Steel 1994; 32, Steel and Barkway 1989; 33, Steel et al. 1987; 34, Truslove 1977; 35, Gendron-Maguire et al. 1993; 36, Mallo 1997; 37, Mallo and Gridley 1996; 38, Rijli et al. 1993; 39, Luettkke et al. 1993; 40, Mann et al. 1993; 41, Wright et al. 1995; 42, Ackley et al. 1994; 43, Hemesath et al. 1994; 44, Hodgkinson et al. 1993; 45, Hughes et al. 1993; 46, Motohashi et al. 1994; 47, Steingrims-son et al. 1994; 48, Tachibani et al. 1992; 49, Copeland et al. 1990; 50, Steel et al. 1992; 51, Zsebo et al. 1990; 52, Deol 1967; 53, Deol 1968; 54, Hosoda et al. 1994; 55, Pavan et al. 1994; 56, Vetter et al. 1996; 57, Letts et al. 2000; 58, Delpire et al. 1999; 59, Deol 1963; 60, Dixon et al. 1999; 61, Flagella et al. 1999; 62, Johnson et al. 1998; 63, Kusakari et al. 1992; 64, Ruckenstein et al. 1993; 65, Trune et al. 1996; 66, Watanabe-Fukunaga et al. 1992; 67, Minowa et al. 1999; 68, Phippard et al. 1999; 69, Phippard et al. 2000; 70, Francis et al. 2000.

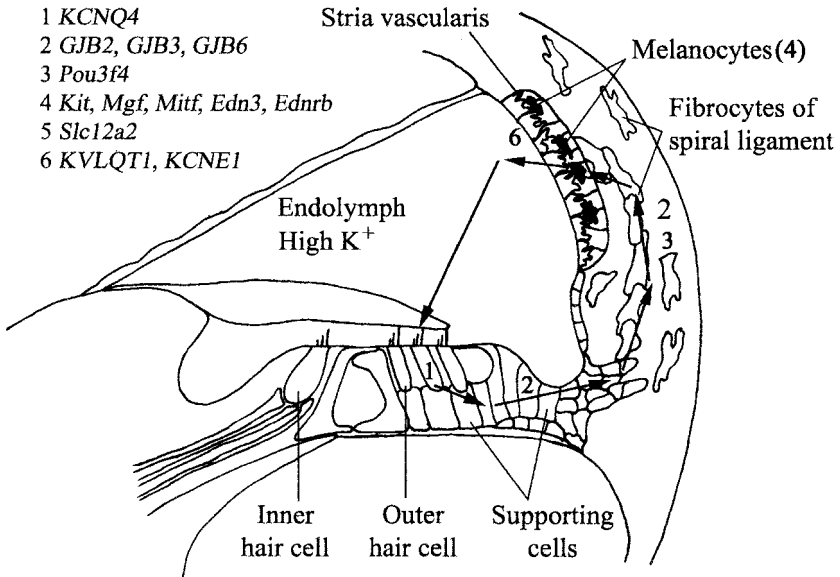


FIGURE 8.4. A schematic representation of the cochlear duct showing the expression pattern of genes thought to be involved in recycling of potassium. *KCNQ4* is expressed in the hair cells. *GJB2*, *GJB3* and *GJB6* are expressed in the supporting cells of the organ of Corti and in the fibrocytes of the spiral ligament. *Pou3f4* is expressed in the spiral ligament. *Kit*, *Mgf*, *Mitf*, *Edn3* and *Ednrb* are all involved in the migration of melanoblasts from the neural crest to the stria and their later differentiation into melanocytes, although these genes are not necessarily expressed in the mature melanocytes. In the marginal cells of the stria vascularis, *Slc12a2* encodes a Na-K-Cl co-transporter located on the basolateral membranes, while *KVLQT1* (*KCNQ1*) and *IsK* (*KCNE1*) are channel proteins located at the luminal surface of the marginal cells. See text for further details.

(Fig. 8.4). Potassium is known to flood into the hair cell when it is stimulated by sound, and it needs to be removed. *KCNQ4* encodes a potassium channel expressed in cochlear hair cells, and has been proposed as a route for the removal of potassium from these cells. Mutation of *KCNQ4* leads to dominantly inherited, progressive hearing loss in humans, but no mouse model has yet been reported (Kubisch et al. 1999). Next, the potassium is thought to be taken up by supporting cells and passed through gap junctions to the spiral ligament (laterally), or the spiral limbus (medially). Mutations in three human connexin genes (*GJB2*, *GJB3* and *GJB6*) expressed in the cochlear duct cause hearing impairment in humans (Denoyelle et al. 1998; Grifa et al. 1999; Kelsell et al. 1997; Xia et al. 1998; Liu et al. 2000), but again no mouse mutants have yet been reported. Evidence that the spiral ligament is important in supporting the generation of the EP comes from a mouse mutant with the *Pou3f4* gene inactivated (Minowa et al.

1999). In these mutants, ultrastructural study of the spiral ligament fibrocytes revealed that they had a reduction in the extent of cell-to-cell connections, and this was associated with a reduced EP measured in scala media. One possible explanation for these findings is that reduced connections between fibrocytes lowered the amount of potassium that could be passed through, in turn affecting the production of the EP by the stria. The *POU3F4* gene is mutated in human X-linked mixed deafness, but it is difficult to imagine that EP could ever be recorded in humans. Thus, making a direct comparison of the cochlear pathology in the two species is difficult. However, a reduced EP could explain the sensorineural component of the mixed hearing impairment observed in these people.

Gap junctions lead up to the basal cells of the stria vascularis. Here, the abundant Na^+ , K^+ ATPase is believed to act in concert with a Na^+ , K^+ , 2Cl^- co-transporter to transport potassium into the marginal cells lining the luminal surface of the cochlear duct. In the *shaker-with-syndactylism* mouse mutant, as well as two knockout mutations of the *Slc12a2* gene, the endolymphatic compartments collapse at around the time of birth, suggesting that it is indeed the co-transporter encoded by this gene that is involved in potassium pumping into marginal cells (Dixon et al. 1999; Delpire et al. 1999; Flagella et al. 1999). Once inside the marginal cell, potassium is thought to pass down its electrochemical gradient into the endolymph via a potassium channel on the luminal surface formed from the products of two genes, *KCNQ1* and *KCNE1*. Either of these genes can be mutated in human Jervell and Lange-Nielsen syndrome (Griffith and Friedman, Chapter 6). Mutations of the mouse orthologues show early collapse of the endolymphatic compartments, supporting the suggestion that these genes are essential for endolymph production (Vetter et al. 1996; Francis et al. 2000). Once in the endolymph, the potassium is available once more for the transduction current into hair cells.

Melanocytes form an essential component of the stria vascularis. The intermediate cells are specialized melanocytes that are scattered between the marginal cells on the luminal side (derived from the otic epithelium), and the basal cells (derived from the mesenchyme). Melanocytes originate in the neural crest during early development, and migrate to the inner ear to ultimately populate the stria vascularis as well as specific locations in the vestibular part of the ear (Steel and Barkway 1989; Steel et al. 1992; Cable et al. 1995). The melanocytes extend many cellular processes that interdigitate with adjacent marginal and basal cell processes. In the young stria they look like typical melanocytes, with extensive dendrites and many pigment-laden melanosomes (Cable and Steel 1991). No particular role for the melanocytes was imagined until the finding that, in mice with no melanocytes in their strias, no EP was generated (Steel et al. 1987). Several mouse mutants are now known to have such a defect, including dominant spotting (*Kit*), steel (*Mgf*), microphthalmia (*Mitf*), piebald (*Ednrb*) and lethal spotting (*Edn3*), and other mutants with white-spotted coats are

thought likely to have similar inner ear defects (see Table 8.4). In some of these mutants, the lack of EP and resulting hearing impairment can be variable: some ears show no EP and no melanocytes present in the stria, while in other ears some melanocytes are detected in the stria, and this invariably correlates with a measurable EP (although it may be smaller than normal). This correlation suggests that the melanocytes are indeed vital for EP generation, and it is not a separate effect of the mutation that leads to the deafness. Exactly what the melanocytes do remains to be determined, but it is unrelated to their ability to synthesize melanin pigment because albino animals have a normal EP and normal, but amelanotic, melanocytes in their strias.

The mutations associated with a lack of melanocytes in the stria also lead to white spotting of the coat, which is not surprising because the melanocytes that populate the skin and hair follicles also migrate from the neural crest during early development. The genes involved encode transcription factors or growth factors and their receptors, all of which are necessary at various stages of melanocyte migration, proliferation, differentiation, or survival. For example, the *Kit* receptor molecule and its ligand *Mgf* are required for melanocyte survival. In mice with mutations in either gene, the melanocyte precursors migrate from the neural crest and can be detected in the mesenchyme surrounding the otic vesicle, but instead of proliferating and moving into the stria, as in normal mice, they appear to die (Cable et al. 1995; Steel et al. 1992).

The association between white spotting of the coat and deafness is seen in many mammals, including humans, and in all cases the deafness can be variable in its penetrance, and can be unilateral. There are several forms of pigmentation anomaly associated with deafness in humans, such as piebald trait due to mutation in the human *KIT* gene, and Waardenburg syndrome, which can result from mutations in *PAX3*, *MITF*, *EDN3* or *EDNRB* (Griffith and Friedman, Chapter 6). It is highly likely that the cochlear defect in these human syndromes is attributable to a lack of melanocytes and a reduced or absent EP, but it is most unlikely that it will ever be possible to test this by measuring the EP in a human.

2.5 Tectorial, Cupular and Otolithic Membrane Defects

A number of mouse mutants have been described with otolithic membrane or cupular membrane defects (see Table 8.5). These mutants are readily detected because of the resulting balance anomalies, leading to the classic shaker-waltzer behaviour of head-tossing, circling and hyperactivity, or alternatively just tilting of the head. These membranes are formed of extracellular matrix material and lie over the sensory hair cells, providing a shearing force to facilitate deflection of hair cell stereocilia. Cupular membranes lie over the hair cells of the cristae, and are deflected when the head moves, causing endolymph to circulate around the semicircular canals,

TABLE 8.5. Mouse mutants with tectorial membrane or otolithic membrane defects

Mutant name	Symbol	Gene	Chromosome	Inheritance	Origin	Key references
Tilted head	<i>thd, ub</i>	NK	1 (59.9)	R	S, R	1, 2
Activating transcription factor 2 KO	<i>Atf2, Creb2</i>	<i>Atf2</i>	2	R	T	3
Pallid	<i>pa, Pallidin</i>	<i>Pallidin</i>	2 (67.6)	R, M	S	4–11
Lethal milk	<i>lm, Znt4, Slc30a4</i>	<i>Slc30a4</i>	2 (69)	R, M	S	12, 13
Maloney sarcoma oncogene KO	<i>Mos</i>	<i>Mos</i>	4 (0)	SD	T	14, 15
Tilted	<i>tl</i>	NK	5 (24)	R	S	16, 17
Deafwaddler, Wriggle mouse	<i>dfw, wri, Pmca2, Atp2b2</i>	<i>Atp2b2</i>	6 (49.5)	R, SD	S	18–20
Sagami, <i>Pmca</i> 2 KO						
Otogelin KO	<i>Otog, Otgn</i>	<i>Otog</i>	7	R	T	21
α tectorin KO	<i>Tecta</i>	<i>Tecta</i>	9 (25)	R	T	22
Mocha	<i>mh, Ap3d</i>	<i>Ap3d</i>	10 (43)	R, M	S	23–25
Cocked	<i>co</i>	NK	11 (46)	R	S	26
Jackson shaker	<i>js</i>	NK	11 (77)	R	S	27–30
Muted	<i>mu</i>	NK	13 (21)	R	S	31
Homeobox, msh-like 2	<i>Msx2, Hox8</i>	<i>Msx2</i>	13 (32)	D	T	32
Torpedo	<i>tpd</i>	NK	15			33
Head tilt	<i>het</i>	NK	17 (4.1)	R	S	34, 35
<i>Col11a2</i> KO	<i>Col11a2</i>	<i>Col11a2</i>	17 (18.5)	R	T	36
Twirler	<i>Tw</i>	NK	18 (3)	SD	S, I?	37, 38
Thyroid hormone receptor α KO	<i>Thra</i>	<i>Thra</i>		E	T	39, 40
Thyroid hormone receptor β KO	<i>Thrb</i>	<i>Thrb</i>		R, E	T	39–42
Careener		NK				43

The table includes the major genes or loci known to be involved in deafness and/or balance defects. Only the key references describing the ear phenotype and the initial identification of the gene are included, so the list is not intended to be comprehensive. The chromosomal localization is given where this is known, with the distance in cM from the centromere given in parentheses. KO, Knock-out. NK, Not known. Under Inheritance column: R, Recessive; D, Dominant; SD, Semidominant; E, Epistatic; M, Maternal effect. Under Origin column: R, Radiation-induced; S, Spontaneous; T, Transgenics and knock-outs; C, Chemical mutagenesis; I, Transgenic Insertional mutations.

References: 1, Erway et al. 1971; 2, Lim et al. 1978; 3, Reimold et al. 1996; 4, Erway et al. 1971; 5, Huang et al. 1999; 6, Lim and Erway 1974; 7, Lyon 1951; 8, Lyon 1955; 9, Schrader et al. 1973; 10,11, Trune and Lim 1983a,b; 12, Erway and Grider 1984; 13, Huang and Gitschier 1997; 14, Propst et al. 1990; 15, Rauch 1992; 16, Bohne and Harding 1997; 17, Ornitz et al. 1998; 18, Kosel et al. 1998; 19, Street et al. 1998; 20, Takahashi and Kitamura 1999; 21, Simmler et al. 2000; 22, Legan et al. 2000; 23, Kantheti et al. 1998; 24, Lane and Deol 1974; 25, Rolfson and Erway 1984; 26, Peterson 1970; 27, Kitamura et al. 1992; 28–30, Kitamura et al. 1991a, b, c; 31, Lyon and Meredith 1969; 32, Hoffman et al. 1995; 33, D. Beier, pers. comm. 2000; 34, Bergstrom et al. 1998; 35, Jones et al. 1999; 36, McGuirt et al. 1999; 37, Lyon 1958; 38, Ting et al. 1994; 39, Rüsche et al. 1998; 40, M Kelley and D Forrest pers. comm. 2000; 41, Campos Barros et al. 1998; 42, Forrest et al. 1996; 43, Chai and Chiang 1962.

which provides information to the brain about head movement. Otolithic membranes lie over the saccular and utricular maculae. They contain small, calcium-rich, crystalline structures called otoliths, which make the otolithic membrane denser than the surrounding endolymph. The gravitational force on the otoliths provides information to the brain about head position. These extracellular membranes are secreted by the underlying epithelium, most likely by the supporting cells.

Several of the mutants described have abnormal otolithic membranes, either small or undetectable, or containing giant otoliths. The defects can be variable among mutants, and can be unilateral (Lim et al. 1978; Trune and Lim 1983a,b). The genes so far identified encode calcium channels and pumps (*Slc30a4*; *Atp2b2*), a component of the intracellular secretory pathway (*Ap3d*), and an extracellular matrix molecule (*Otog*) that appears to be required to attach the membrane to the underlying epithelium. Defective otolithic membranes have been described in mutations of two transcription factors (*Atf2* and *Msx2*). Such defects may be secondary to gross inner ear malformations, and there are probably many examples of inner ear malformations with otolithic or cupular defects that are not specifically described in the literature. Mutants with specific otolithic or cupular membrane defects do not necessarily have impaired cochlear function, and no humans with mutations in the same genes have been described yet.

The tectorial membrane is the extracellular matrix of the cochlea, and is secreted by the supporting cells of the organ of Corti as well as the cells of Kölliker's organ during development. Kölliker's organ later regresses to form the inner spiral sulcus. Abnormalities of the tectorial membrane have been described in many different deaf mouse mutants. However, the structure is notoriously susceptible to histological artefact during preparation, and when underlying hair cells degenerate, it loses some of its attachment points, so care must be taken in interpreting these observations, since they may not involve a primary tectorial membrane defect. However, recently four different mutants have been described that do seem likely to involve primary tectorial membrane defects and associated hearing impairment. The *Col11a2* knock-out and the *Otog* knock-out mutations have both been described with subtle ultrastructural anomalies of the tectorial membrane, suggesting that the molecules encoded by these genes are probably components of this membrane (Simmler et al. 2000; McGuirt et al. 1999). A targeted mutation of *Tecta*, in contrast, leads to major ultrastructural abnormalities of the tectorial membrane, which is not surprising because the α -tectorin molecule encoded by *Tecta* was identified previously as a major component of the membrane (Legan et al. 2000). Lastly, double mutants for two thyroid hormone receptors, *Thra* and *Thrb*, show more severely elevated thresholds than the *Thrb*-deficient single mutant, and preliminary analysis indicates the presence of some malformation of the tectorial membrane in the double mutant (M Kelley and D Forrest, personal communication 2000).

Two of these tectorial membrane genes are known to be involved in human hereditary deafness; they are *COL11A2* and *TECTA*. *TECTA* is mutated in some forms of both dominant and recessive nonsyndromic deafness (DFNA8/12 and DFNB21), while *COL11A2* underlies the nonsyndromic DFNA13 as well as syndromic deafness in Stickler/OSMED syndrome (Griffith and Friedman, Chapter 6). It seems likely that tectorial membrane abnormalities underlie the hearing impairment seen in these forms of human deafness. People with mutations of the thyroid hormone receptor *THRB* show resistance to thyroid hormone and often have associated hearing impairment (Brucker-Davis et al. 1996; Refetoff et al. 1967). It is not clear whether these people are hearing-impaired because of a tectorial membrane defect, or because of some other cochlear defect resulting from delayed development.

2.6 Neural Defects

There is an increasing number of mouse mutants reported to have specific defects in the peripheral or central auditory pathways (Tables 8.6 and 8.7). One group of molecules, the neurotrophins and their receptors, appears to be essential for the survival of inner ear neurons during development. Both cochlear and vestibular afferent neurons arise from cells that delaminate from the early otic vesicle, migrate from the epithelium to form the cochleovestibular ganglion, extending dendrites back towards sensory hair cells in the inner ear and axons that connect with the central auditory and vestibular nuclei. Mice with knockouts of the brain-derived neurotrophic factor gene (*Bdnf*), or its receptor (*Ntrk2*), have no surviving afferent innervation of the cristae of the semicircular canals and a much reduced afferent supply to the maculae and to cochlear outer hair cells. In contrast, in mice with the neurotrophin-3 gene (*Ntf3*) or the corresponding receptor gene (*Ntrk3*) disrupted, the afferent innervation of the cochlea is severely reduced and there is minor loss of vestibular neurons. In mice that have both receptors (*Ntrk2* and *Ntrk3*) or both neurotrophins (*Bdnf* and *Ntrk3*) inactivated, there is a complete loss of afferent innervation to the inner ear. The effects of these mutations have been reviewed in some detail recently (Fritzsch et al. 1997b, 1999b and Table 8.6 for references).

Other mutations can lead to abnormal development of the inner ear ganglia. Mutations in *Pou4f1* or *Ap2* lead to reduced or abnormal cochleovestibular ganglia (McEvelly et al. 1996; Zhang et al. 1996; Schorle et al. 1996), and knock-out of the neurogenin1 gene, *ngn1*, causes a complete absence of all afferent, efferent and autonomic innervation of the inner ear (Ma et al. 1998; Fritzsch et al. 1999a).

Efferent fibres arise from the neural tube in the hindbrain region, and the autonomic innervation of the inner ear originates in the superior cervical ganglion, a neural crest derivative (Fritzsch et al. 1997b). There are several mutants that affect the development of the efferent system. The

TABLE 8.6. Mouse mutants with peripheral neural defects

Mutant name	Symbol	Gene	Chromosome	Inheritance	Origin	Key references
Myelin protein zero KO	<i>Mpz</i> , <i>P0</i> , <i>Mpp</i>	<i>Mpz</i>	1 (92.4)	R	T	1
GATA binding protein 3 KO	<i>Gata3</i>	<i>Gata3</i>	2 (7)	R	T	2
Nociceptin receptor KO				R	T	3
Brain-derived neurotrophic factor KO	<i>Bdnf</i>	<i>Bdnf</i>	2 (62)	SD	T	4–7
Nicotinic acetyl choline receptor $\alpha 9$ KO	<i>Acra9</i>	<i>Acra9</i>	5 (41)	R	T	10
Homeobox A1 KO	<i>Hoxa1</i>	<i>Hoxa1</i>	6 (26.3)	R, E	T	11–15
Neurotrophin 3 KO	<i>Ntf3</i> , <i>Ntf3</i>	<i>Ntf3</i>	6 (61)	R	T	6, 16, 17
Neurotrophin receptor C KO	<i>Ntrk3</i> , <i>trkc</i>	<i>Ntrk3</i>	7 (39)	SD	T	6, 18–20
Trembler	<i>Tr</i> , <i>Pmp22</i>	<i>Pmp22</i>	11 (34.5)	D, SD	S	21–24
Neurogenin1 KO	<i>Ngn1</i> , <i>Neurod3</i>	<i>Neurod3</i>	13 (35)	R	T	25,26
Neurotrophin receptor B KO	<i>Trkb</i> , <i>Ntrk2</i>	<i>Ntrk2</i>	13 (36)	R, SD	T	6, 18–20
<i>Pou4f1</i> KO	<i>Pou4f1</i> , <i>Brn3a</i> , <i>Brn3.0</i>	<i>Pou4f1</i>	14	R	T	27
<i>erbB4</i> receptor tg	<i>erbB4</i>	<i>erbB4</i>		D	T	28

The table includes the major genes or loci known to be involved in deafness and/or balance defects. Only the key references describing the ear phenotype and the initial identification of the gene are included, so the list is not intended to be comprehensive. The chromosomal localization is given where this is known, with the distance in cM from the centromere given in parentheses. KO, Knock-out. NK, Not known. Under Inheritance column: R, Recessive; D, Dominant; SD, Semidominant; E, Epistatic; M, Maternal effect. Under Origin column: R, Radiation-induced; S, Spontaneous; T, Transgenics and knock-outs; C, Chemical mutagenesis; I, Transgenic Insertional mutations.

References: 1, Martini et al. 1995; 2, Pata et al. 1999; 3, Nishi et al. 1997; 4, Bianchi et al. 1996; 5, Ernfors et al. 1994; 6, Ernfors et al. 1995; 7, Jones et al. 1994; 8, Schorle et al. 1996; 9, Zhang et al. 1996; 10, Vetter et al. 1999; 11, Chisaka et al. 1992; 12, Gavalas et al. 1998; 13, Lufkin et al. 1991; 14, Mark et al. 1993; 15, Rossel and Cappechi 1999; 16, Fariñas et al. 1994; 17, Frittsch et al. 1997a; 18, Frittsch et al. 1995; 19, Minichiello et al. 1995; 20, Schimmang et al. 1995; 21, Adlkofer et al. 1995; 22, Suter et al. 1992; 23, Wang et al. 1995; 24, Zhou et al. 1994; 25, Ma et al. 1998; 26, Frittsch et al. 1999a; 27, McEvilly et al. 1996; 28, Rio et al. 1999.

contralateral efferent supply to the inner ear arises from rhombomere 4 of the developing hindbrain, and this projection across the midline is abnormal or absent in *Hoxb1* or *Gata3* mutants (Studer et al. 1996; Pata et al. 1999). The nicotinic acetyl choline receptor $\alpha 9$ subunit is normally expressed in sensory hair cells, and in mice with mutations of this gene the

pattern of synapses is altered on outer hair cells, with a single large terminal instead of many small endings on each hair cell (Vetter et al. 1999). The efferent supply of outer hair cells is believed to permit suppression of cochlear responses, and this physiological feature is absent in the $\alpha 9$ mutants (Vetter et al. 1999).

Several neurological mutants show specific defects of cell types within the central auditory pathways. The nervous, lurcher, Purkinje cell degeneration, and staggerer mutants all have specific cartwheel cell defects in the dorsal cochlear nucleus, and in reeler mutants the dorsal cochlear nucleus is disorganized with reduced numbers of granule cells (Berrebi and Mugnaini 1988; Berrebi et al. 1990; Martin 1981).

TABLE 8.7. Mouse mutants with central auditory system defects

Mutant name	Symbol	Gene	Chromosome	Inheritance	Origin	Key references
GATA binding protein 3	<i>Gata3</i>	<i>Gata3</i>	2 (7)	R	T	1
Osteopetrosis, Colony stimulating factor 1 KO	<i>op, Csf1 Csfm</i>	<i>Csf1</i>	3 (51)	R	S	2
Maloney sarcoma oncogene KO	<i>Mos</i>	<i>Mos</i>	4 (0)	SD	T	3, 4
Pax5 KO	<i>Pax5, BSAP</i>	<i>Pax5</i>	4 (20.7)	SD	T	5, 6
Reeler	<i>Reln, rl</i>	<i>Reln</i>	5 (8)	R	S, C, I	7–9
Homeobox A1 KO	<i>Hoxa1</i>	<i>Hoxa1</i>	6 (26.3)	R, E	T	10–14
Lurcher	<i>Lc, Grid1, cpr, ho</i>	<i>Grid1</i>	6 (29.6)	SD	S	15,16
Deafwaddler, Wrinkle mouse	<i>dfw, wri, Pmca2,</i>	<i>Atp2b2</i>	6 (49.5)	R, SD	S	17–19
Sagami, <i>Pmca</i> 2 KO	<i>Atp2b2</i>					
Quivering	<i>qv, dyn, lnd</i>	NK	7 (14.5)	R	S	20–23
Albino	<i>c, Tyr</i>	<i>Tyr</i>	7 (44)	R	S, R	24–26
Nervous	<i>nr</i>	NK	8 (8)	R	S	27
Myodystrophy	<i>myd, fg</i>	NK	8 (33)	R	S	28
Staggerer	<i>sg, Rora</i>	<i>Rora</i>	9 (36)	R	S	15,29
<i>Foxb1a</i> KO	<i>Fkh5, Mf3, Foxb1a, Twh</i>	<i>Foxb1a</i>	9 (41)	SD	T	30
Orthodenticle homologue 1 KO	<i>Otx1</i>	<i>Otx1</i>	11 (12)	R, E	T	31–34
Trembler	<i>Tr, Pmp22</i>	<i>Pmp22</i>	11 (34.5)	D, SD	S	35–37
Homeobox B1 KO	<i>Hoxb1</i>	<i>Hoxb1</i>	11 (56)	R, E	T	38–41
Hypothyroidism	<i>hyt, pet, Tshr</i>	<i>Tshr</i>	12 (37)	R	S	42–44
Purkinje cell degeneration	<i>pcd</i>	NK	13 (37)	R	S	15
Wabblers-lethal	<i>wl</i>	NK	14	R	S	45

TABLE 8.7. *Continued*

Mutant name	Symbol	Gene	Chromosome	Inheritance	Origin	Key references
Orthodenticle homologue 2 KO	<i>Otx2</i>	<i>Otx2</i>	14 (19)	SD, E	T	34, 46, 47
Protein dehydrogenase KO	<i>Prodh, Pro1</i>	<i>Prodh</i>	16 (10.7)	R	T	48
Quaking	<i>qk</i>	NK	17 (5.9)	R	S, C	49–51
Shiverer	<i>Mbp, shi,</i> <i>mld</i>	<i>Mbp</i>	18 (55)	R	S	52
Fibroblast growth factor 8 KO	<i>Fgf8, Aigf</i>	<i>Fgf8</i>	19 (45)	R	T	53
Nociceptin receptor KO				R	T	54

The table includes the major genes or loci known to be involved in deafness and/or balance defects. Only the key references describing the ear phenotype and the initial identification of the gene are included, so the list is not intended to be comprehensive. The chromosomal localization is given where this is known, with the distance in cM from the centromere given in parentheses. KO, Knock-out. NK, Not known. Under Inheritance column: R, Recessive; D, Dominant; SD, Semidominant; E, Epistatic; M, Maternal effect. Under Origin column: R, Radiation-induced; S, Spontaneous; T, Transgenics and knock-outs; C, Chemical mutagenesis; I, Transgenic Insertional mutations.

References: 1, Pata et al. 1999; 2, Michaelson et al. 1996; 3, Propst et al. 1990; 4, Rauch 1992; 5, Reimer et al. 1996; 6, Urbánek et al. 1994; 7, D'Arcangelo et al. 1995; 8, Hirotsune et al. 1995; 9, Martin 1981; 10, Chisaka et al. 1992; 11, Gavalas et al. 1998; 12, Lufkin et al. 1991; 13, Mark et al. 1993; 14, Rossel and Cappechi 1999; 15, Berrebi et al. 1990; 16, Zuo et al. 1997; 17, Kosel et al. 1998; 18, Street et al. 1998; 19, Takahashi and Kitamura 1999; 20, Bock et al. 1983; 21, Deol et al. 1983; 22, Horner and Bock 1985; 23, Horner et al. 1985; 24, Conlee et al. 1991; 25, Creel et al. 1983; 26, Moore et al. 1988; 27, Berrebi and Mugnaini 1988; 28, Mathews et al. 1995; 29, Hamilton et al. 1996; 30, Wehr et al. 1997; 31, Acampora et al. 1996; 32, Acampora et al. 1998; 33, Acampora et al. 1999; 34, Morsli et al. 1999; 35, Adlkofer et al. 1995; 36, Suter et al. 1992; 37, Zhou et al. 1994; 38, Gavalas et al. 1998; 39, Goddard et al. 1996; 40, Rossel and Cappechi 1999; 41, Studer et al. 1996; 42, Bertoni et al. 1993; 43, O'Malley et al. 1995; 44, Stein et al. 1994; 45, Harman et al. 1954; 46, Acampora et al. 1995; 47, Matsuo et al. 1995; 48, Gogos et al. 1999; 49, Ebersole et al. 1996; 50, Shah and Salamy 1980; 51, Sidman et al. 1964; 52, Fujiyoshi et al. 1994; 53, Meyers et al. 1998; 54, Nishi et al. 1997.

Other mutants have been reported to have a reduced or absent inferior colliculus (*Fkh5*, Wehr et al. 1997; *Fgf8*, Meyers et al. 1998; *Otx2*, Matsuo et al. 1995; *Pax5*, Urbánek et al. 1994), or an enlarged inferior colliculus, as in *Otx1* mutants (Acampora et al. 1996). This does not necessarily correlate with abnormal gross function (Reimer et al. 1996), although detailed analyses have not yet been carried out in any of these mutants. Prolonged interpeak intervals of brainstem-evoked potentials suggesting abnormal central auditory system function have been reported in the myodystrophy (*myd*, Mathews et al. 1995), shiverer (*Mbp*, Fujiyoshi et al. 1994), quaking (*qkI*, Shah and Salamy 1980) and osteopetrosis (*Csf1*, Michaelson et al. 1996) mutants, but the structural basis of these functional defects is not known. The quivering mutant has normal gross auditory-system anatomy,

and normal cochlear response thresholds, but thresholds are raised when measured from central nuclei, suggesting a defect of central origin (Bock et al. 1983; Horner and Bock 1985).

Lastly, two mutants show specific central auditory-system dysfunction. The nociceptin-receptor knock-out mouse shows normal thresholds, but an impaired ability to recover from intense sound exposure. The expression of this receptor molecule around the crossed olivocochlear bundle, which supplies the contralateral efferent neurons to the cochlear hair cells, suggests the receptor may act by modulating efferent activity (Nishi et al. 1997). Proline dehydrogenase (*Prodh*) mutants also show normal thresholds, but attenuated pre-pulse inhibition, a phenomenon whereby the behavioural response to a sound is moderated by prior exposure to a pre-pulse. This observation suggests a defect in central processes of sensorimotor gating (Gogos et al. 1999).

It is very likely that many other mouse mutants, particularly those with generalized neurological defects, will be shown to have specific auditory system anomalies, but most of them have not yet been analysed in any detail. Central auditory system anomalies are highly likely to be found in any cases of peripheral hearing impairment because of the importance of normal sensory input to refining the neural circuitry of the brain during development. However, specific central auditory system defects are relatively rare in humans, and there are no clear examples wherein comparison with any of the mouse models described above is possible. However, pre-pulse inhibition has been described as a feature of schizophrenia, so any mouse mutants showing this feature may be of particular interest to psychiatric research.

3. Summary

There is now a large number of mouse mutants with hearing and/or balance defects available for investigating the reasons for the impairment, and these mutants will all contribute to our growing understanding of the complexity of deafness. Many more mouse mutants are candidates for involvement of the auditory system, but their hearing has not yet been investigated in any detail. Some of these are listed in additional tables available at the Web site accompanying this chapter (Steel 2000). However, comparison of the chromosomal locations of these mutations causing deafness in the mouse with the locations of known human deafness mutations reveals that there are many human loci for which no mouse model has yet been discovered. Two major mutagenesis programs are ongoing in Europe, at Neuherberg, Germany and Harwell, UK, and new deaf mouse mutants are being isolated from these screens to help to fill the gap between human deafness and mouse models (Nolan et al. 2000). Large-scale, genome-wide mutagenesis programmes are starting in other countries too, including the

US, so there will soon be many more mutants available. Deafness is one of the most heterogeneous diseases known in humans, and study of the many deaf mouse mutants will help unravel the molecular basis of the pathology, an essential first step towards a rational approach to treatment.

Acknowledgments. This work was supported by the UK Medical Research Council, the European Commission, and Defeating Deafness. We thank the people who have kindly allowed us to include their data prior to publication, including Drs. Ken Johnson, Yin Zheng, Verity Letts, Wayne Frankel, Jo Cable, Rachel Hardisty, Matthew Kelley, Douglas Forrest and David Beier, and Donna Fekete for kind permission to reprint Figure 8.1.

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9

Genetic Counseling for Deafness

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1. Introduction

Genetic counseling is an important process through which families receive information regarding the cause of a hereditary condition in a family member, its management, its inheritance, and other medical or psychological implications. Genetic counseling has been defined as “a communication process that deals with the human problems associated with the occurrence, or risk of occurrence, of a genetic disorder in a family” (Ad Hoc Committee on Genetic Counseling 1975). Instead of focusing on prevention of genetic disease, genetic counseling emphasizes informed decision-making by families based on information provided to them about the genetic conditions that may be present. Genetic counseling is specific to the needs of individual families. The emphasis is on effective communication and non-directive provision of information. While some families are concerned about the prevention of a genetic condition in future children, other families seek only to gather information on the condition as it relates to medical, psychological, or educational concerns.

2. The Importance of Genetic Counseling for Deaf Children and Adults

Genetic factors account for a significant proportion of congenital or early-onset deafness, and they are also thought to account for many cases of later-onset hearing loss (Sill et al. 1994). There are major benefits of genetic counseling for individuals and families with deafness, including families with young children with hearing loss and deaf or hard of hearing adults. In particular, most children with hearing loss can benefit from a genetic evaluation, particularly with emerging improvements in the ability to use genetic testing to confirm a specific diagnosis. In addition to providing information about the cause of hearing loss, other medical implications, and chance of recurrence, an effective genetic counselor can recognize the

emotional state of the family and assist them, if necessary, with the process of grieving, adjustment, acceptance of the diagnosis, and decision-making. In considering the needs of families during genetic counseling, the “functional/cultural” status of the family as well as the “audiologic” status is taken into account. Families with several generations of deaf members who consider themselves to be part of the “Deaf community” may be just as interested and in need of genetic counseling as hearing parents who have just received the diagnosis of hearing loss in a young child.

2.1 The Importance of Referral

Professionals who work with families with deaf or hard of hearing children or adults can be responsible for informing the family about the benefits of genetic counseling and can guide them in determining at what time a referral may be appropriate. The urgency for genetic counseling is influenced by many factors, including the emotional adjustment of the family, the presence and seriousness of additional medical complications and the reproductive concerns, if any, of the family. It is just as important for professionals to refer families in which there is only an isolated case of hearing loss as it is to refer those families that have many affected members.

A deaf child with a negative medical history, a normal physical examination, and no family history of hearing loss, is likely to have a genetic etiology of deafness (Keats and Berlin, Chapter 1). Definitive information about the exact genetic cause of hearing loss is often possible using genetic screening and testing methods that have recently become available. Evaluation by a clinical geneticist, in addition to genetic testing, can rule out the presence of a genetic syndrome. The family can be provided with specific information about inheritance and recurrence risks and can be given access to a number of research protocols or clinical tests available for confirming a diagnosis of hereditary deafness. The genetic counselor can also discuss with the family specific misconceptions they may hold regarding the cause of hearing loss. This type of discussion is often helpful in alleviating the guilt parents may feel regarding hearing loss in a child. It can also assist the parents in acceptance of the hearing loss, and help them to move on with their lives so that they can provide support and advocate for the educational and social needs of their child.

Referrals for genetic counseling should also be considered for adults with hearing loss. Deaf and hard of hearing adults can benefit greatly from the genetic counseling process (Arnos et al. 1992). As individuals reach the age at which they consider their reproductive options, many become very curious about the cause of their hearing loss and the chances of passing that condition to their children. Genetic counseling is beneficial to adults who have progressive types of hearing loss, or who develop later-onset hearing loss, as well as to those individuals who were born deaf and often view their deafness as a cultural difference rather than a disability.

For the culturally deaf, most of whom have congenital, severe to profound sensorineural deafness, their identities have been shaped by their use of a common language (American Sign Language in the United States) and the ways in which they have been educated and socialized. Many of these deaf individuals have been educated together in residential or mainstream programs for the deaf. The size of the American Deaf community is currently estimated to be several hundred thousand people (Padden and Humphries 1988). Approximately 90% of these individuals marry another deaf person (Schein 1989). Many deaf couples would prefer to have deaf children and are eager to find out about the cause of their own deafness (Jordan 1991; Arnos et al. 1991). While these individuals may be stigmatized by past experiences of medicalization of their deafness and by misunderstandings of the goals of genetic counseling, the availability and sensitivity of genetic counselors to their special needs has greatly improved, giving more and more deaf couples very positive and beneficial experiences with genetic counseling. Both deaf and hard of hearing adults can benefit from an exact diagnosis of the etiology of hearing loss, information about any associated medical or physical features (syndromes), access to research protocols or clinical tests, and reproductive information.

It may be appropriate for some families or individuals to be referred a second time for genetic counseling. If there is a significant change in the family history (i.e., the birth of another child with a hearing loss) or in the medical history (i.e., a deaf child develops night blindness), another referral is indicated. A deaf adult who was initially evaluated as a child may now wish to talk with a genetic counselor about the implications of the earlier diagnosis for family planning. Lastly, because of the rapid pace with which this field is growing, a second or subsequent referral may be appropriate in order to discuss new advances in genetic testing.

3. The Process of Genetic Counseling

As shown in Table 9.1, the process of genetic counseling involves the collection of different types of information, and proceeds through several different steps. The genetics evaluation is often performed by a team of professionals, which includes a clinical geneticist (a medical doctor) and a genetic counselor, among others. Once a family or individual has been referred for evaluation and counseling, the genetic counselor will work with them to assess their needs and the purpose of the evaluation, and to collect preliminary information regarding medical, family and audiologic history. This information will often be collected over the phone, or the family may be asked to fill out a history form prior to their visit. The physical examination and discussion with the family will occur over one or two face-to-face visits with the clinical geneticist and the genetic counselor.

TABLE 9.1. The genetic counseling process

-
1. Collection of family history
 2. Collection of medical history
 3. Review of audiometric information
 4. Physical examination by certified clinical geneticist
 5. Additional medical studies or referral to specialists
 6. Screening for genes for deafness/referral to research protocols
 7. Discussion of diagnosis, inheritance pattern, prognosis and treatment options
 8. Follow-up and other referrals
-

3.1 Family History

Family history information is critical in making a diagnosis of the cause of deafness. The mode of inheritance of the hearing loss or clues to the presence of a syndromic form of deafness is often revealed through the careful collection of an accurate family history. Important information includes the health and hearing status of siblings, parents and other close family members, the possible occurrence of consanguinity (blood relationship) between the parents of a child with hearing loss, and ethnic background. Questions that reveal possible syndromic forms of deafness focus on the occurrence of eye disease; pigmentary changes of the skin, hair, or eyes; structural malformation of the ears or face; skeletal variations; and other problems such as kidney malformations or heart disease (Griffith and Friedman, Chapter 6).

3.2 Medical History

A medical history profile can be assembled by obtaining copies of records documenting birth history, chronic health problems, or other serious illnesses that may be related to the etiology of the hearing loss. Medical information may be collected on other family members as well as the person or persons with hearing loss. Such information provides useful details about a possible syndromic form of deafness in the family or may assist in ruling out environmental (nongenetic) causes of the hearing loss.

3.3 Audiologic History

Information regarding age of onset, and degree and severity of hearing loss is typically collected for all family members by the genetic counselor through the interview process. Subsequent documentation of these parameters of hearing loss may be obtained by requesting copies of previous evaluations, or asking family members to have a comprehensive evaluation by a certified audiologist.

3.4 Physical Examination

A clinical geneticist certified by the American College of Medical Genetics is qualified to perform a physical examination as part of the genetics evaluation. The clinical geneticist is trained to recognize specific traits, features, and aspects of the family history that may lead to a specific diagnosis of the cause of hearing loss. This person assimilates information from the family history, medical history, and results of the physical examination in making a diagnosis. A genetic examination may contain aspects that are similar to other physical examinations, such as listening to the heart and looking in the ears; but a genetic evaluation also includes more observations (of eye color, ear shape, etc.) and measurements (of the head circumference, eyes, etc.). Since many deaf individuals who are referred for genetic evaluation have syndromic forms of hearing loss, the clinical geneticist will pay special attention to the presence of any physical features such as goiter; pigmentary abnormalities of the eye, hair, or skin; severe myopia; fainting spells or heart disease; abnormalities of the external ears, face, or skeletal structures; or evidence of kidney disease or eye disease.

3.5 Additional Medical Studies or Referral to Specialists

The clinical geneticist may recommend evaluations by other specialists or additional laboratory tests based on the findings. For example, a person with hearing loss who is found to have ear pits or tags will be referred for a renal ultrasound when a diagnosis of branchio-oto-renal syndrome is considered. A deaf child who is found to have difficulty with night blindness or who has pigmentary retinopathy will be referred to an ophthalmologist. In fact, a formal ophthalmologic evaluation is recommended even in children who appear to have isolated sensorineural hearing loss because of the educational significance of the progressive loss of vision associated with Usher syndrome. Children who have a history of syncopal episodes would be referred to a cardiologist to rule out prolonged QT, associated with Jervell and Lange-Nielsen syndrome. An adult with a progressive hearing loss and cataracts may be referred for a urinalysis to check for hematuria when Alport syndrome is suspected. A chromosome study may be recommended for a deaf child who has multiple malformations involving other organ systems. There are many other tests or referrals that may be indicated based on the findings of the clinical geneticist.

3.6 Screening for Genes for Deafness/Referral to Research Protocols

Knowledge of the molecular genetics of hearing loss is growing rapidly. The chromosomal locations of dozens of genes causing both syndromic and non-

syndromic hearing loss have been determined by linkage analysis (Mueller, Van Camp, and Lench, Chapter 4). Testing for most of these genes is now available on a research basis and, for some, on a clinical basis.

Genetic testing may be appropriate in a variety of situations and for several reasons. When a syndromic form of deafness is suspected, genetic testing may be able to confirm or rule out a particular syndrome. Hearing parents with one or more deaf children may be interested in testing to identify the cause of deafness in these children. Subsequently, they may want to know about their options for carrier testing for their hearing children and prenatal diagnosis for future pregnancies. When both members of a couple have nonsyndromic autosomal-recessive deafness, they may desire testing to find out whether their chances to have deaf children are near 0% or 100%.

It is important for the genetic counselor to be aware of and be prepared to discuss appropriate clinical tests and research protocols with families. For clinical testing, genetic counselors should be able to explain what type of sample is needed and how much, the cost of the test, and the turn-around time for results. In evaluating a research protocol, genetic counselors should know the inclusion criteria for a study and be able to answer families' questions, such as: "What does participation require of me?" "Is there any cost to me?" and "Will I get results from the study?" It is also critical that genetic counselors be involved in conveying the results of testing to the family. A person who receives the results of genetic testing may have many questions regarding carrier testing for other members of the family, reproductive options, and treatment. The identification of a specific form of hereditary deafness may also be a very emotional time for the person or the family. It is important that a genetic counselor be the primary point of contact or part of a team of individuals responsible for providing this information to the family.

3.7 Discussion of Diagnosis, Inheritance Pattern, Prognosis, and Treatment Options

During a genetic counseling session or sessions following the genetic evaluation, the individual or family will be given complete information regarding the diagnosis, mode of inheritance, prognosis, reproductive implications, treatment options, options for prenatal diagnosis, and other recommended options for support or follow-up. Particular attention is paid to providing this information in a manner that is sensitive to the individual's needs and the emotional state of the individual or family. The decision-making process regarding reproductive and treatment options for hereditary deafness may be strongly influenced by the cultural identification and psychosocial needs of hearing parents of a deaf child or deaf adults who identify with the Deaf community. In the non-directive and supportive atmosphere provided by a sensitive and responsive genetic counselor, individuals or families are

encouraged to absorb as much medical and genetic information as they can understand, consider possible courses of action, and begin the decision-making process for any choices that must be made. Arrangements for professional sign language or oral interpreters will be made for deaf and hard of hearing children or adults who require these services. It is important in genetic counseling situations that family members not be used as interpreters. Genetic counselors are also given training in providing information in a non-directive fashion that is sensitive to different reproductive preferences or cultural differences. This is an especially important consideration when providing counseling to deaf individuals (Arnos et al. 1991). In providing non-directive counseling for deaf couples, for example, genetic counselors must be aware that word choice can convey cultural bias. Use of the word “chance” instead of “risk” for having a deaf or hearing child is neutral terminology. The use of such terminology enhances openness and communication because it implies that assumptions are not being made about how the couple may feel about having deaf or hearing children. Other examples include the use of the terms “deaf,” “hard of hearing,” and “hearing” when referring to family members instead of the medicalized terms “affected” and “unaffected.”

The genetic counseling session may take place during one visit or may require several visits. Some families are seen for follow-up counseling to assess changing medical aspects, psychosocial concerns, or new advances in genetic screening and testing. Because of the differing needs of families, genetic counseling sessions are unique to every family in terms of content, approach, and types of support provided. Many families can benefit from referrals to support groups, communication specialists, or educators, particularly parents with a young deaf child who need guidance regarding communication and educational options and opportunities to network with other parents of deaf children. Genetic counselors often work together with audiologists, social workers, and psychologists to assist families in obtaining this type of information.

4. Recurrence Risks/Empirical Risk Estimates

The genetic counselor will often need to rely on empirical risk estimates when informing families about recurrence risks. For hearing parents whose first child is deaf due to an etiology that cannot be determined after genetic evaluation, and who otherwise have a negative family history, an empirical risk estimate of 9% would be given. A deaf couple would be given an empirical risk of 10% for their first child to be deaf, given that an etiology could not be determined in either of them. Empirical risks are also helpful in many other situations (Bieber and Nance, 1979). However, the ability to provide accurate information regarding recurrence risks to hearing parents of a single child with hearing loss and to adults with hearing loss

has been greatly assisted by the availability of genetic tests for common forms of genetic deafness. In the future, there will be a decreasing need for genetic counselors to rely on empirical risks when providing information to families.

5. The Future is Now: Genetics Technology and the Genetic Counseling Process

As described in several of the preceding chapters, advances in the identification and characterization of genes for hearing loss are occurring very rapidly. These technologies are beginning to have a profound effect on the genetic counseling process. Because these advances are relatively new, many genetic counselors are only beginning to learn about the availability of clinical diagnostic tests and research-based tests available for hearing loss. For some conditions, carrier testing and prenatal diagnosis is now possible and is being offered by some programs.

It is important that geneticists, as well as other professionals involved in the health care of individuals with hearing loss, consider the ethical implications associated with the utilization of genetics technology. As pointed out by Nance (1993), the genetics/medical community, deaf and hard-of-hearing people, and parents of young deaf children may have widely divergent views regarding the appropriate use of genetics technology. While there are many benefits of the availability of genetic testing to diagnose both syndromic and nonsyndromic forms of deafness, there are many ethically challenging situations that can arise. At what point should the decision be made to offer genetic testing on a clinical basis rather than a research basis? At what point should carrier testing and prenatal diagnosis be offered? Should hearing parents at risk for a deaf child be given the option of prenatal diagnosis and termination of the pregnancy? Should these parents first be given detailed information about the effects (both negative and positive) of deafness upon the lives of children? Should deaf parents who desire to have deaf children be given this option through genetic testing, prenatal diagnosis, and selective termination of a hearing fetus? Although this situation may only rarely arise, it is within the realm of possibilities. Now that it has become evident that connexin 26 mutations are very common causes of congenital deafness, deaf people may seek testing for the purposes of avoiding having deaf children or, alternatively, improving their chances of having deaf children. Either of these goals could be accomplished simply by the deliberate choice of a marriage partner without or with mutations in the connexin 26 gene. How do we best encourage an open dialogue between the medical/genetics community and deaf and hearing consumers regarding these issues? Should we encourage this type of dialogue at all? These and other issues need to be considered by

any program that provides genetic testing for deafness. Because of the existence of the deaf community and the cultural identity of this group, these discussions are particularly relevant regarding hereditary deafness.

A recent study completed in the United Kingdom by Middleton and colleagues (1998) examined the attitudes of a group of deaf individuals towards genetic testing for deafness. A group of 87 deaf individuals filled out a structured, self-completion questionnaire that included items to assess preference for having deaf or hearing children, feelings about new discoveries in genetics, and whether genetic testing devalued deaf people. Results indicated predominantly negative attitudes towards genetics and genetic testing for deafness. The results of the survey provided evidence that many members of the deaf community are threatened by genetics technology and feel that it is inappropriate to use such technology for selective termination of deaf fetuses. While some of the survey participants stated a preference for deaf children, most said that they were not interested in prenatal diagnosis for deafness. Of those who said they would consider prenatal diagnosis, 29% said that they would prefer deaf children. Almost half of the individuals surveyed said they felt that the potential use of genetic testing devalued deaf people, and 55% felt that genetic testing would do more harm than good. A much larger study of attitudes of deaf people is in progress by this group; however, it is clear that, as the authors state, "some deaf persons may prefer to have deaf children and may consider the use of genetic technology to achieve this." There are certain to be great differences in attitudes among deaf and hard of hearing people which may be strongly influenced by their degree of hearing loss, age at onset, hearing status of their parents, their education and socialization, and chosen method of communication. While some feel it would be wrong to deliberately propagate deafness in their children, others feel that it is only natural to desire children who will be deaf like themselves.

6. Case Examples

The following cases demonstrate some of the medical and psychosocial issues that may arise during genetic counseling for deaf individuals. These cases also illustrate some applications of genetic testing to the clinical situation. A deaf couple planning a family, a college-aged girl with a syndromic form of deafness, and a hearing couple with deaf children will be discussed.

6.1 *Case 1*

M.S. (a 25-year-old white female) and D.S. (a 27-year-old white male) are a profoundly deaf couple who are self-referred to learn more about the cause of their deafness and their chances of having deaf or hearing

children. Both have been deaf since birth, attended residential schools for the deaf, and consider themselves to be members of the Deaf community. They indicated that having healthy children is most important to them. Having healthy, deaf children would be their preference.

6.1.1 Medical History

M.S.'s mother reports that she had rubella during the fourth month of her pregnancy with M.S. M.S.'s medical history is otherwise unremarkable. Pregnancy, delivery, and medical history are unremarkable for D.S.

6.1.2 Audiologic Findings

Based on audiometric studies, M.S. has a profound sensorineural hearing loss with pure-tone averages of 101 on the right and 100 on the left. D.S. also has a profound sensorineural hearing loss bilaterally, with pure-tone averages of 108 on the right and 95 on the left. Neither M.S. or D.S. has obtained any benefit from amplification.

6.1.3 Family History

M.S. has three siblings, two of whom are hearing, and one of whom has a high-frequency hearing loss (Fig. 9.1). The brother with the hearing loss has

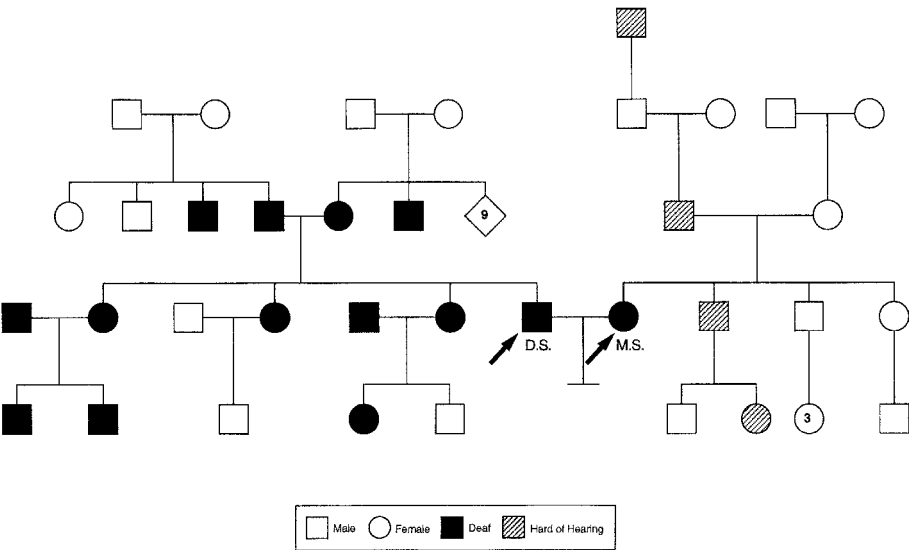


FIGURE 9.1. Family history of M.S. and D.S. M.S. was diagnosed with congenital rubella syndrome and D.S. with nonsyndromic deafness. Their chance of having deaf children is low.

a daughter with a high-frequency hearing loss. M.S.'s other nieces and nephews are hearing. M.S.'s mother is hearing, and her father has a high-frequency hearing loss. His paternal grandfather was said to be "very hard of hearing." No audiometric information to document degree or progression of the hearing loss is available for these relatives.

D.S. is one of four deaf siblings and is the product of deaf parents (Fig. 9.1). One of his sisters married a deaf man from a deaf family and has two deaf sons. Another sister married a deaf man with a family history of deafness and has one deaf and one hearing child. The third sister married a hearing man of deaf parents and has one hearing son. D.S.'s parents each have a deaf brother and other hearing siblings. They were both born to hearing parents.

6.1.4 Physical Examination

M.S. is a petite woman who is said to be smaller than her siblings. Funduscopic examination revealed mottled coloration of her retinas. There was no audible heart murmur. The rest of the examination was within normal limits. D.S.'s physical examination was within normal limits with no evidence of a genetic syndrome.

6.1.5 Clinical Diagnosis and Recommendations

M.S. was diagnosed as having congenital rubella syndrome (Table 9.2). D.S. was diagnosed as having nonsyndromic, autosomal recessive, sensorineural hearing loss. It is also likely that an autosomal-dominant form of nonsyndromic sensorineural hearing loss is present in M.S.'s family.

6.1.6 Genetic Counseling Issues

Genetic counseling in this situation would include a discussion of congenital rubella syndrome (CRS) and why this is the likely cause of M.S.'s deafness. There is a 50% chance that M.S. has also inherited the gene for autosomal-dominant high-frequency hearing loss from her father, but that it is masked by the CRS. If she has inherited the gene, there is a 50/50 chance with each pregnancy that she will pass on that gene to her child.

TABLE 9.2. Congenital rubella syndrome

Fetal exposure to a maternal rubella infection may cause changes in several organ systems, most commonly the ear, heart, and eyes. Infants may have a sensorineural deafness. They may have a congenital heart defect, usually patent ductus arteriosus, pulmonary stenosis, ventricular septal defect, or atrial septal defect. Common eye findings in congenital rubella syndrome (CRS) are cataracts and congenital glaucoma. Other features associated with CRS include small stature, jaundice, enlarged liver and spleen, small head, learning disabilities or mental retardation, anemia, low platelet count, changes in the hardening of the long bones, and a rash.

During the genetic counseling session, a detailed description of autosomal-recessive inheritance would be provided. All of D.S.'s future children will be carriers of the gene causing his deafness. Because D.S. and M.S. have different types of hearing loss, their chances of having congenitally deaf children appear to be low. However, D.S. has an apparently common form of hearing loss as is evident by the fact that his mother and father and one sister married someone with the same type of hearing loss. Therefore, it is possible that M.S. may be a carrier of the same type of deafness as D.S., in which case they would have a 50/50 chance of having deaf or hearing children with each pregnancy.

This couple's perception of deafness and their desires for deaf or hearing children would be explored. They may have never considered the possibility of having a hard of hearing child and may be surprised to learn about this chance. In addition, they may have suspected that their chance to have deaf children was high because D.S. has a deaf family.

They would be referred to a genetics center performing molecular genetic studies of nonsyndromic deafness. Depending on whether the testing at that center is performed on a clinical or research basis and whether D.S. has a form of deafness for which there is currently a test, this testing may or may not aid this couple in decisions about family planning.

6.1.7 Overview of Case 1

This case demonstrates the complexity of counseling deaf couples and the potential ability of molecular testing to aid genetic counselors in providing the most accurate recurrence risk information possible. There are at least three different types of hearing loss in this family. Identifying the gene that caused D.S.'s deafness and testing M.S. to see whether she carries that gene will allow a genetic counselor to determine whether the couple's chance to have a deaf child is the same as the general population's chance of 1 in 1000, as suspected based on the clinical information, or whether that chance is actually 50%. In addition, without molecular testing, there is no way to know prior to M.S.'s having children whether she has inherited a gene from her father for high-frequency hearing loss. This case also addresses the issue of deaf people's perceptions of their chance to have deaf children and their desires to have deaf or hearing children, which vary greatly from individual to individual.

6.2 Case 2

S.K. is a 23-year-old, profoundly deaf, white female who is self-referred to learn more about why she is deaf and why she has different colored eyes. She was born deaf and attended residential schools for the deaf for most of her life. S.K. currently is not married and does not plan to have children in the near future, although she is curious about her chance to have deaf or hearing children, should she decide to have children in the future.

6.2.1 Medical History

S.K.'s prenatal, birth, and early medical history are unremarkable. Past medical history is significant only for asthma.

6.2.2 Audiologic Findings

S.K. has a profound bilateral sensorineural hearing loss with pure-tone averages of 93 on the right and 100 on the left. She obtains no benefit from amplification.

6.2.3 Family History

As shown in Figure 9.2, S.K. has one sister who is deaf, has blue eyes, and patchy hypopigmentation of her skin. Her father is hearing, and her mother has a profound unilateral hearing loss. Her mother has blue eyes and was born with a white forelock. She was grey by age 30. Her brother and sister, S.K.'s uncle and aunt, are hearing. The brother has blue eyes, and the sister, brown. Their father was deaf in one ear, had green eyes, and was grey by age 25. Their mother was hearing. S.K.'s father comes from a hearing family in which everyone has brown eyes.

6.2.4 Physical Examination

Physical examination by the clinical geneticist revealed a young woman with average height and stature. She had auburn hair with a few strands of

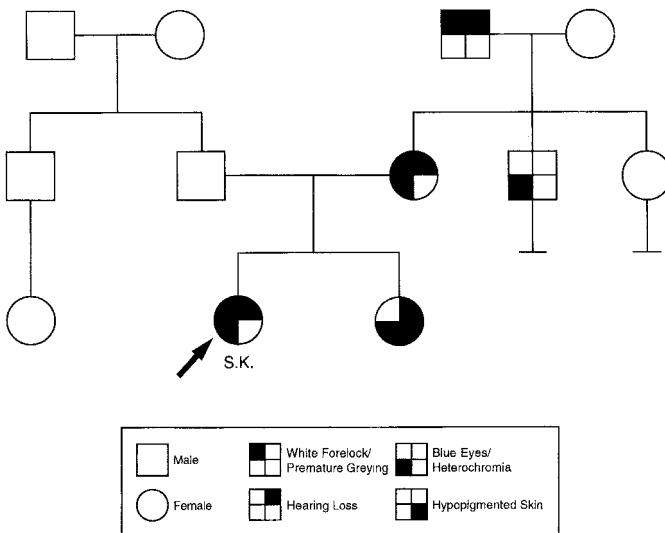


FIGURE 9.2. Family history of S.K., who has Waardenburg syndrome type II.

gray hair just above the forehead. Her left eye was blue and her right, brown. There was no dystopia canthorum. Her nose was well formed. Her skin was evenly pigmented.

6.2.5 Clinical Diagnosis and Recommendations

A diagnosis of Waardenburg syndrome type II was made. (see Table 9.3.) Because of the potential implications for family planning, genetic evaluation of other family members (especially S.K.’s aunt and uncle) would be recommended to confirm or rule out Waardenburg syndrome.

6.2.6 Genetic Counseling Issues

During this genetic counseling session, the features of Waardenburg syndrome (WS) would be discussed. A clear explanation would be given to S.K. to help her understand that WS has caused both her deafness and different colored eyes and has also caused other pigmentary changes in her family members (white forelock, skin hypopigmentation and early graying of the hair). A detailed explanation of autosomal-dominant inheritance with variable expression would take place. S.K., as well as anyone else in her family with Waardenburg syndrome, would have a 50% chance of having a child with WS. There is a 50% chance that a person with WS type II will be deaf. Therefore, S.K. has a 25% chance with each pregnancy to have a deaf child. S.K. would be advised to return for genetic counseling when she marries or is planning to have children. She has indicated that she prefers to marry another deaf person and would be interested in finding out about their chances of having a deaf child.

6.2.7 Overview of Case 2

This case demonstrates the importance of making an accurate diagnosis of a syndrome. The associated features of Waardenburg syndrome are rarely of medical significance and may be subtle; someone not trained in genetics

TABLE 9.3. Waardenburg syndrome

Waardenburg syndrome (WS) is characterized by sensorineural hearing loss that may be mild to profound and unilateral or bilateral, heterochromia (often one blue and one brown eye) or bright blue eyes, congenital white forelock or premature greying, and hypopigmented skin. In rare instances, individuals with Waardenburg syndrome have serious medical complications, such as Hirschsprung’s disease or spina bifida. There are two common forms of Waardenburg syndrome, types I and II, which are distinguished by the presence or absence of dystopia canthorum and by the frequency of hearing loss. Waardenburg syndrome type I is characterized by dystopia canthorum. About 20% of people with WS type I have a hearing loss. People with Waardenburg syndrome type II have normally spaced eyes, and about 50% have a hearing loss. All forms of Waardenburg syndrome are inherited as a dominant trait.
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may overlook them. Had the diagnosis of Waardenburg syndrome type II been missed, one might have assumed the chance for S.K. to have deaf children was greater or less than the actual chance of 25%. This case also addresses the notion of the family, and not the individual, as the patient. A diagnosis of a genetic condition in an individual may have profound health or reproductive implications for other family members.

6.3 Case 3

T.R. (a 23-month-old Hispanic female) and her brother J.R. (a 4-month-old Hispanic male) were referred for a genetic work-up by their audiologist because of recent identification of sensorineural hearing loss.

6.3.1 Medical History

T.R. and J.R.'s prenatal, birth, and medical histories are unremarkable. T.R.'s mother reported that T.R. has had an ophthalmological examination and CT scan of the temporal bones since the hearing loss was diagnosed. Both were reported to be within normal limits.

6.3.2 Audiologic Findings

Because of concerns about speech development, T.R. had an ABR at age 18 months, which indicated a sloping, moderate to severe sensorineural hearing loss bilaterally. Follow-up sound field testing is consistent with the ABR results. Pure-tone averages are 60 on the right and 55 on the left. J.R. had an ABR at age 2 months because of his sister's hearing loss. A bilateral, moderately severe sensorineural hearing loss is suspected. Both children are being fitted for hearing aids.

6.3.3 Family History

As shown in Figure 9.3, all other family members, including the parents and a half-brother, are hearing. The family history is unremarkable.

6.3.4 Physical Examination

T.R.'s physical examination revealed slight micrognathia, but was otherwise unremarkable. J.R.'s physical examination was within normal limits with no evidence of a genetic syndrome.

6.3.5 Clinical Diagnosis and Recommendations

A diagnosis of autosomal-recessive, nonsyndromic, sensorineural hearing loss was made. Records from T.R.'s examinations following the diagnosis of hearing loss were requested to confirm that they were within normal limits.

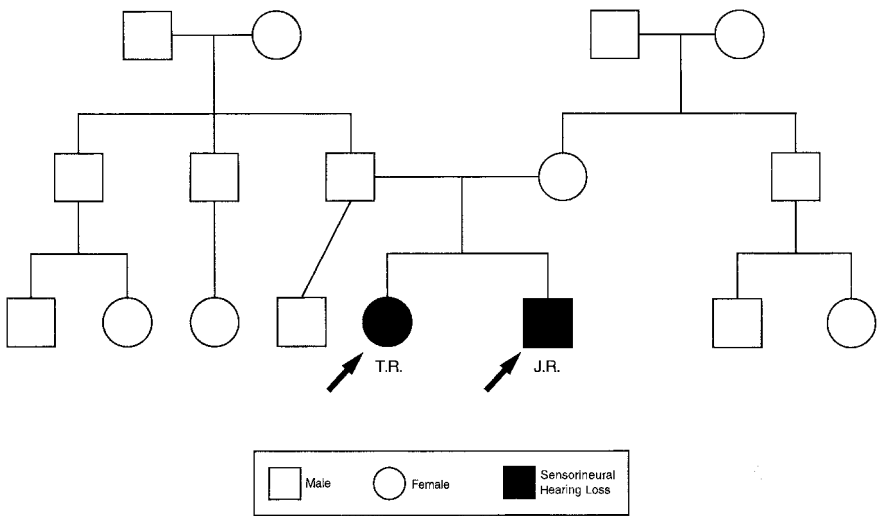


FIGURE 9.3. Family history of T.R. and J.R. who were diagnosed with autosomal-recessive, nonsyndromic hearing loss.

6.3.6 Genetic Counseling Issues

Genetic counseling in this situation would include a discussion of autosomal-recessive inheritance. Any future children T.R. and J.R.'s parents may have would have a 25% chance of having a hearing loss. The chance for T.R. and J.R. to have children with hearing loss depends on how frequent the gene causing their hearing loss is in the population, whether they marry a person with a hearing loss, and the cause of their mates' hearing loss, if any. Both T.R. and J.R. would be encouraged to seek genetic counseling again at the time they are considering marriage or children.

Issues regarding how the parents feel about their child's hearing loss would be explored. Feelings of guilt or blame ("It's my fault my children are deaf") may be present. Referral to a support group or counseling may be beneficial. The parents may also have many questions about educational and language choices for their children, and appropriate referrals would be made.

This family would be referred to a genetics center performing molecular genetic studies of nonsyndromic deafness. Families agree to participate in research for different reasons. Although this family may receive no apparent immediate benefit from participation in research, it may be of benefit to family members in years to come. Identification of the gene for hearing loss present in the family may assist the parents with dealing with their feelings about passing deafness on to their children. It may also allow

genetic counselors to provide information about possible progression of the hearing loss, which may be important in making educational and language choices. Identification of a gene for nonsyndromic deafness in these children would also alleviate any concerns the parents may have about the possibility of future medical complications associated with syndromic forms of deafness and eliminate costly testing done to diagnose these complications.

6.3.7 Overview of Case 3

This case demonstrates the issues facing hearing parents with deaf children, which differ from those facing deaf adults. These parents may be grieving the loss of their “perfect” child. They may also feel unequipped to care for a deaf child and overwhelmed by the number and importance of decisions they must make. Deaf adults have often been deaf their entire lives and never experienced a loss and therefore likely do not require emotional support.

This case also demonstrates that hearing couples with young deaf children may seek molecular genetic testing for reasons different from an adult deaf couple (such as the couple in Case 1). In addition to identifying a specific form of hearing loss and allowing for more accurate genetic counseling, genetic testing may eliminate the need for some costly, time-consuming medical evaluations, and provide information about possible progression of the hearing loss.

7. Summary

Genetic counseling is the process of providing information to families about a genetic condition such as deafness in an atmosphere that is non-directive, supportive, and sensitive to the special needs of individuals. Professionals who provide health care and services to deaf and hard of hearing children and adults are essential to the referral process. Genetic counseling includes the collection of medical and family history information, a physical examination by a certified clinical geneticist, referral for genetic diagnostic tests offered on a research or clinical basis, and referral and follow-up for any medical conditions that are found to be associated with the deafness. Families are also provided with detailed information regarding the cause of the deafness, treatment options and reproductive implications, and are given support and guidance in making decisions about these options. The availability of genetic testing for many hereditary forms of deafness has improved the ability of genetic counselors and clinical geneticists to provide accurate information to families. However, there are many ethical implications of this type of testing yet to be explored. These considerations are particularly important given that some deaf people identify themselves as being part of a separate cultural group based on their deafness.

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